PDU Final Report

October 19, 1995

Run number: P950425CF
Dates of run: 4/25/95 to 5/8/95
Feedstock: Corn fiber

1.0 Executive *Summary*

The Process Development Unit (PDU) was operated with a corn fiber feedstock for approximately 10 days using the Amoco Pretreatment Reactor (APR), the seed fermentation train, fermentation support equipment, and the main 9000-L fermenters. The run utilized the yeast strain L1400 to ferment glucose to ethanol in the 9000-L fermenters using the simultaneous saccharification and fermentation (SSF) process. No back-end unit operations (e.g., distillation, centrifugation) were used during the run. However, operation of the centrifuge was checked after the run using corn-fiber fermentation broth.

Operation of the plant was improved because of the experience gained from the previous run. However, there were not enough resources available between runs to fix the feed valve and level control problems noted during the March run. The APR operated for over nine days with only minor interruptions, but was finally shut down because of wear and corrosion problems. The conversion of glucan to glucose was approximately 50%, and 60%—70% of the xylans were converted to xylose in pretreatment. The overall carbon balance for two APR samples were 76% and 73%. A material balance was performed on a SSF sample from near the end of the run, when ethanol concentrations were their highest. The yields were 24.6-, 9.8-, and 14.1-g/g C6 sugar consumed for ethanol (theoretical yield for ethanol yield is 51.0), acetic acid, and lactic acid, respectively. A high level of contaminate cells consumed much of the glucose giving a low ethanol yield (approximately 50%) and high by-product yields. Achieving higher ethanol concentrations in future PDU runs will require reduced contaminate concentrations and by-product yields.

This run successfully achieved integrated and continuous operation of the APR and main fermentation train. Solids levels in SSF were 25%, a significant improvement over run specifications. The seed train fermenters also performed well and produced uncontaminated inoculum for the main fermenters. Several problems still need to be resolved and fixed including automating level control in the main fermentecs, automating of the feed addition systems for corn steep liquor, enzyme, and inoculum, fixing of the fermentation exhaust gas flowmeters, and adding measurements of caustic flow rates to the fermenters. Future operation will also look at contamination sources and control of contamination to reduce by-product yields and increase ethanol production.

2.0 Introduction

The primary purpose of this run was to operate the PDU for approximately 10 days to prove sustained mechanical operation of the APR and fermentation equipment. The run served to identify and investigate solids levels, and control and contamination problems. Although downstream equipment (i.e., distillation and centrifugation) was not ready to run, this run integrated operation of the APR with the SSF train. Continuous SSF was conducted on pretreated corn fiber in three 9000-L fermenters. The fermentation used the yeast strain L1400 chat was

grown in the seed fermentation train using the 20-L, 160-L, and 1450-L fermenters.

2.1 Pilot Plant Configuration

This section provides a brief description of the PDU as background on the overall process. A simplified process flow diagram for the PDU without the APR is shown in Figure 1. The figure shows the overall flow path and equipment in the PDU. The process begins with feedstocks being loaded into a storage hopper (SH-120) and then continuously fed by a weigh belt (SA-150) to a mill (ND-110) by a pneumatic system. The milled particles are separated from the air stream by a cyclone (FG-110) and fed to another weigh belt (SA-120). This belt controls feed rates to the rest of the plant. The feedstock is conveyed (SC-120) to a mixer (MX-250) and mixed with acid and water. The acidified biomass is fed to a high-temperature, high-pressure pretreatment reactor (MX-204) by a plug feeder (MX-270) that creates a impervious biomass plug. Temperature, acid concentration, and residence time are controlled in the reactor to achieve adequate pretreatment. The pretreated material is then cooled by flashing to the flash tank (MX-205). This material is then pumped (P-205) to the first 9000-L fermenter (V-455A), or alternatively, it can be pumped to a hold tank (V-210A) for storage,

In the first 9000-L fermenter, pretreated biomass is combined with inoculum, cellulase enzyme (from V-321), and corn steep liquor (from V-420). The microorganism is started in a small shake flask and successively transferred to a larger shake flask, the 20-L fermenter, 160-L fermenters (V-445A/B), 1450-L fermenters (V-450A/B), and finally to the seed hold tanks (V-465A/B) to await addition to the first 9000-L fermenter. Once the first fermenter in the train (V-455A) is filled, overflow fermentation broth from V-455A is pumped to the next 9000-L fermenter (V-455B) in the train. Each fermenter receives continuous feed and its level is controlled to maintain a desired residence time. Exhaust gas from the fermenters is sent to a scrubber (T-460) to remove volatile organics and odors. The beer well (V-510) receives and holds spent fermentation broth from the last fermenter (V-455D).

Fermentation broth in the beer well can be pumped to either the neutralization tank (V-602) for pH adjustment and then disposal, or pumped to the distillation column (T-501) for removal of the ethanol. Partially purified ethanol from the column is condensed and sent to the ethanol storage tank (V-506). The stream from the bottom of the column is cooled (in E-506) and sent to a feed tank (V-601). This material is then pumped to the centrifuge (FF-610) to remove the remaining solids, which drops into the cake tank (V-611). The solids are then sent to disposal through V-602. The liquid fraction from the centrifuge is collected in the centrate tank (V-610); this liquid can then be sent to disposal (through V-611 and V-602) or to the sterilization tank (V-256). Sterilized liquid is held in a feed tank (V-257) and can then be used as make-up water for the process.

For this run, the APR with its own feeder and acid and water delivery systems were used in place of the feed handling equipment and pretreatment reactor described above. Spent fermentation broth was sent to the neutralization tank for disposal. The distillation equipment was not operated and the centrifuge was operated after the run on broth remaining in the 9000-L fermenters.

3.0 Pilot Plant Operations

Operation of the plant began on April 25 and continued until May 8 using the APR and fermentation equipment. The PDU feed handling and pretreatment equipment were not utilized in this run. Operating conditions were decided upon before the run and these conditions are presented in the next sections. Additionally, a run history and significant operational notes are presented.

3.1 Procedures and Operating Conditions

3.1.1 Feed Handling/Pretreatment Operating Conditions

Corn fiber was obtained from a local corn wet-milling facility (Golden Technologies, Inc., Johnstown, CO) and stored in plastic-lined, 55-gal drums in a refrigerated trailer. Initially, the APR was fed at a rate of 150 wet lb/h, but the rate was gradually increased to 170 wet lb/h by 50 hours into the run. The goal was to feed the SSF train 1 dry ton/d of corn fiber, but 150 lb/h (38%0 solids) was the highest rate ran prior to this experiment; this was only 68% of the desired rate. Originally, the goal was to increase the APR throughput to about 220 lb/h (at 38% solids) if possible, which is equivalent to 1 dry ton/d. However, after two days it was decided to suspend further increases in favor of seeking steady state more quickly and limiting the duration of the run. One unexpected event was the dryer corn fiber received from Golden Technologies for this run (42% solids for the first batch and 45% solids for the second batch). Since the APR is weight controlled, this led to a dry feed rate at 92% of the target by the end of the run.

The acid concentration was meant to be 1.0 wt% of the slurry. There were some problems in controlling the acid concentration and flow during the test, and as a result the addition rate was higher than anticipated, about 1.3 wt% for much of the run. Coupled with the lower-than-anticipated water content of the feedstock, this gave an acid concentration in the water phase 50% higher than design.

3.1.2 Fermentation Operating Conditions

Operating conditions for the seed train are shown in Table 2. L1400 was grown by successive transfers from a small shake flask to a larger shake flask, and then to the 20-L, 160-L, and 1450-L fermenters, respectively. There was no pH control in the shake flask. pH was controlled with 3.0 molar NaOH in the 20-L and 160-L fermenters and with 50% NaOH in the 1450-L fermenter. Inoculum from the 1450-L fermenter was transferred to the seed hold tanks to await addition to first 9000-L fermenter. The seed tanks were agitated at 50 rpm, maintained at a gauge pressure of 0.33 bar, and cooled with circulating water.

Fermentation conditions in the 9000-L fermenters are also presented in Table 2. Corn steep liquor (CSL) was not used in this run and enzyme additions were made to only the first 9000-L fermenter. A 10% (w/w) inoculum was also added to first 9000-L fermenter from the seed hold tanks. pH was controlled using 50% NaOH. Level was controlled in the 9000-L fermenters to maintain a residence time of 24 h in each vessel. Solids concentrations were 24%—28% after dilution of the pretreated corn fiber by enzyme and inoculum. Composition and flow rate of off-gas was not measured.

3.2 Run History

A time line for this run is shown in Figure 2. Operation of the APR began at 14:00 on April 28 and continued until 22:00 on May 7. Minor shutdowns of the APR occurred throughout the run because of power failures, feeder problems, and acid line problems.

Seed train fermenters were operated as shown on the time line. Inoculum was first prepared in the 20-L fermenter on April 26 and was operated thereafter as needed to maintain a viable seed supply. The 1450-L fermenter was successfully operated in the "draw and fill" mode to provide inoculum to the first 9000-L fermenter (V-455A). Operation of the first 9000-L fermenter began on April 28 and continued until May 8. When each of the 9000-L fermenters reached their target levels (corresponding to a 24 h residence time), transfers began to the next fermenter in the continuous train.

Table 2. Fermentation Operating Conditions For Seed Train and 9000-L Fermenters

Operating Condition	Flask #1	Flask #2	20-L	160-L	1450-L	9000-L
Temperature ("C)	30	30	30	30	30	30
Agitation (rpm)	150[a]	150"	150	100	75	50
pH	5.0	5.0	5.0	5.0	5.0	5.0
Gauge Pressure (bar)	-	-	0.33	0.33	0.33	0.33
Airflow (vvm)	-		0.5	0.5	0.25	0.03[b]
Residence Time (h)	8[c]	8[c]	8[c]	12"	12'	24[d]
Media:						
Glucose (%)	2	2	2	2	2	[e]
Peptone (%)	2					
Yeast Extract (%)	1					-
CSL (%)		I	1	1	1	-
Antifoam (corn oil, mL/L)	-	-	0.5	0.5	0.5	
Enzyme (IU/g cellulose)	-	-				10

[a] laboratory shaker agitation
[b] air added to maintain a positive pressure in vessels
[c] typical incubation times
[d] per 9000-L vessel
[e] substrate was pretreated corn fiber

3.3 Operational Notes

The following is a list of significant operational notes and problems that occurred during this run.

3.3.1 Feed Handling/Pretreatment

- Power to the APR was lost through operator error at 09:00 on May 2. Power was quickly restored and the unit was brought back on-line and began feeding to the first fermenter within two hours of losing power.

- A plant-wide shutdown occurred on May 3 at 11:00 because of a power interruption from the utility

company. Operation of the APR was restored within an hour

- No problems were encountered with transfer of pretreated feed from the pump at the end of the APR to the fermenters. However, lime for neutralization of the acid was initially added just before the pump, but after deposits (possibly gypsum) were found in the pump, the lime addition point was switched to the first 9000-L fermenter.

-

- On May 5 at 08:30, the APR was shut down for approximately one hour because of an acid line Plug.

-

-

3.3.2 Fermentation

- As noted in the last PDU run report, a high initial solids loading in the first 9000-L fermenter caused mixing problems at startup of this vessel. To avoid this situation in the current run, the fermenter was initially filled with approximately 1250 kg of sterile water. This diluted the solids and allowed adequate mixing to occur until enzyme hydrolysis thinned the broth.

- Continuous inoculum addition was controlled by manual adjustment of the feed valve, because a functional flowmeter was not available. This should be automated once a flowmeter is in place.

- Enzyme additions were also made by manual control of the feed valve, again because no flowmeter was available. However, this did not allow accurate flow control, and on several occasions (May 4, 00:15 and May 6, 15:30) most of the contents of the enzyme tank were dumped into the fermenter.

- Fermenter level was controlled by manually adjusting the speed of the fermenter pumps. Level was maintained within ± 6% of the selected level (3200 L). This should be automated once a reliable level measurement is available.

- Switching the lime addition point to the first 9000-L fermenter (normally added to the flash tank) for this run caused plugging problems because of the long line and a low flow rate. Lime addition immediately downstream of the APR in a mixer led to rapid buildup of white precipitate in the mixer, and was discontinued for fear of forming a plug. Therefore, lime addition was eliminated and 50% NaOH was added to the first 9000-L fermenter (V-455A) for neutralization of

sulfuric acid and pH control.

- Contamination was detected on April 30 in the main fermenters. Contamination issues are discussed later in this report.

- In an attempt to control contamination, the pH in the 9000-L fermenters was lowered from 5.0 to 4.0 for 2 hours and then brought up 4.5 for the rest of the run. It was hoped that a lower pH would reduce the growth rate of a bacterial contaminant.

- The plant-wide power failure of May 3 caused loss of computer control of the 9000-L fermenters, which shut off cooling water to the 9000-L fermenters. Temperature rose in the fermenters because of the addition of hot pretreated material. The consequences of this are discussed in the fermentation results.

- A white chemical deposit was found on the upper impeller blades (above liquid level) of the agitator of the first 9000-L fermenter. Chemical analysis identified this material as sodium carbonate and it was probably produced from the reaction of NaOH (used for pH control) dropping through the vessel headspace with carbon dioxide produced during the fermentation.

4.0 Key Results

The following sections presents key results from operation of the pretreatment and fermentation equipment.

4.1 Pretreatment

Figure 3 shows component concentrations (monomeric sugars and acetic acid) in the pretreated slurry liquors during the run. Concentrations of furfural and HMF were detected in only trace quantities and so are not reported. Time zero for all run data is March 26 at 9:00 and is the time of inoculation of the first 9000-L fernenter. Conversions decreased out to a run time of 43 hours because the increasing feed rate, which decreased the residence time. After 43 hours the APR operating conditions were held at constant conditions with minor variations in the temperature. The data need further analysis to see if product properties correlate to operating conditions, but some conclusions can be drawn from the raw data. Product variability was unacceptably high. There were a number of short shutdowns that perturbed steady-state operation of the APR, and some drift in the operating conditions, and changes in the feed corn fiber. Nonetheless, the variation in feed quality must be brought under better control.

Figure 4 shows monomeric to total soluble sugar ratios (monomeric divided by total soluble sugar) for a few liquor samples. The first four samples show higher monomer levels than the last hvo samples (99-h and 123-h). These particular samples were from the middle part of the run (Figure 3), where there is a noticeable drop in xylose, glucose, and acetic acid concentrations indicating less conversion.

The composition of the two corn fiber lots used in this run are shown in Table 3. Each lot was obtained on different days from the Golden Technologies, Inc. corn wet-milling plant. The data is an average of two independent measurements of the composition for each lot. Each lot sample was a composite of samples taken throughout the run. The precision is indicated by the %RPD, which is Lot 1 was used from April 28 until 09:00 on May 3, then Lot 2 was used until the end of the run.

Using the data in Table 3 and compositional analysis of the pretreated corn fiber, Tables 4 and 5 present

material balances, yield data, and monomeric to total sugar ratios for two APR samples. The results were nearly identical for each of the samples with nearly 50% of the glucans converted to monomeric or oligomeric glucose and 65% conversion of the xylans. The target conversions are about 65% of the glucans and 85% of the xylans. The overall carbon recoveries were 76% and 74%, respectively, compared to overall recoveries of 102% and 95% in the previous run (P950319CF), which used the Sunds reactor for pretreatment. In general, the balance on glucans and xylans is rather poor at 77% to 80%. The APR does not have flow meters and so continuous flow rate data are not obtained, which may be the cause for the poorer mass balances.

Table 3. Composition of Corn Fiber Lots in Percent[a]

Component	Corn Fiber Lot 1		Corn Fiber Lot 2	
	Composition (%)	%RPD	Composition (%)	%RPD
Glucose[b]	41.6	1.2	39.9	5.0
Xylose	21.2	9.4	21.5	4.2
Galactose	7.7	7.8	7.6	3.9
Arabinose	12.8	2.3	12.2	2.5
Mannose	0.0	0.0	0.0	0.0
Lignin	7.8	27.9	8.5	5.6
Acid Soluble Lignin	7.8	13.2	8.1	28.8
Ash	0.9	5.5	0.9	2.2
Protein	10.4		11.6	
Starch	24.9		25.6	
Total Solids	42.4		45.9	

[a] Based on total sugars
[b] Starch is also included in the glucose number

4.2 Fermentations

Concentrations of glucose (monomeric), lactic acid, acetic acid, and ethanol, and temperature and pH in each of the three 9000-L fermenters are shown in figures 5 through 7. During the first two days of the run in the first 9000-L fernenter (Figure 5), ethanol concentration increased as expected with low concentrations of lactic and acetic acid and glucose. However, after three days it is apparent that a contaminant is consuming the sugars and producing lactic and acetic acid and causing a decrease in ethanol concentration. At approximately 100 hours into the run, the temperature in the fermenter increased to 47°C (because of the power failure, as discussed in section 2.3.2). This probably killed most of the microorganisms in the fermenter, and so glucose concentration rose while lactic and acetic acid and ethanol concentrations all fell. Also note that during this time, the pH in the fermenters was changed from 5.0 to 4.5. After temperature control was restored glucose was again rapidly consumed to produce ethanol, since the fermenter was continually inoculated with fresh yeast and a large fraction of the contaminant was probably killed by the high temperature. The concentration of lactic and acetic acid continued to remain low until near the end of the run, when the concentrations of these components began to increase, indicating increasing contaminant

Table 4. PDU Pretreatment **Material** Balance - APR

Run #: P950425CF
Date: 4/30/95 APR-041
Time: 9:00

Rin Conditions: **Temperature (C) :** Flash Tank Temp. (C): 96

Acid Concentration (%): (Liquid **Phase)**

Input Data:

Feed flow Rote (SA-206 kg/h):	72.7	**Feed Solids Concentration (%):**	42.0
Steam (kg/h):	70.9	V-201 **Acid Concentration (%):**	7.3
Acid **Flow Rate** (FT-201-1, kg/h):	17.0		
Valve Water (kg/h):	7.3	Hydrolyzate **Insoluble Solids (%):**	10.8
Water to Pump (kg/h):	8.5		
Flash Vapor (kg/h):	16.5		

Calculated Results:

Solids Solubilized (%):	70.24
Monomer/Total Sugar Ratio (%):	
Glucose:	38.66
Xylose:	48.76
Galactose:	70.48
Arabinose:	79.84
Mannose:	

Carbon Balance: Pretreatment

	Unpretreated		Pretreated												
	Dry Feed	*Carbon In*	*In Solids*			*In Liquid*				*In Flash*			*Total*	
Component	(% dry weight)	(C-mole/h)	(% dry weight)	(C-mole/h)	(% C In Feed)	(g/L mon.)	(g/L total)	(C-mole/h)	(% C In Feed)	(g/L)	(C-mole/h)	(% C In Feed)	(C-mole/h)	(% C In Feed)
Glucose	41.6	423.033	43.5	131.663	31.1	31.7	02	204.988	48.5				338.079	79.9
Mannose	0	0.000	*0*	0.000	*0.0*	***0.0***	0	0.000	**0.0**				0,000	
Galactose	7.7	70.302	4.85	14.680	18.7	7.4	10.5	26.248	33.5				40,928	52.3
Xylose	21.2	215.584	10.1	30.570	14.2	27.5	56.4	140.992	65.4				171,562	79.6
Arabinose	12.8	130.164	4.5	13.620	10.5	30.1	37.7	94.245	72.4				107.865	82.9
Acetic Acid							4.6	11.499		0.4	0.2		11.718	
Formic Acid							0	0.000						
Lactic Acid							1.4	3.500					3.500	
Lignin	16.5	240.779	26.3	14.231	47.4		10.7	38.384	15.9				**152,615**	63.4
Furfural							***0***	*0,000*	0.0	***0.0***	*0.0*	*0.0*		
HMF							0.4	1.428	0.3					
Total	*90.8*	***1087.861***	*82.7*	*304.764*	*28.0*			***521.285***	47.9		*0.2*	*0.0*	***826.268***	

Ignores protein

C- RECOVERY: **75.95%**

Table 5. PDU Pretreatment Material Balance - APR

Run #: P950425CF
Date: 5/6/95 APR-059
Time: 9:00

Run Conditions: Temperature (C): Flash Tank Temp. (C): 96
Acid Concentration (%): (Liquid Phase)

Input Data:

Feed How Rate (SA-206 kg/h):	77.3	Feed Solids Concentration (%):	45.0
Steam (kgfn):	17.6	V-201 Acid Concentration (%):	7.4
Acid Flow Rate (FT-201-1, kg/h):	19.3		
Valve Water (kg/h):	7.3	Hydrolyzate Insoluble Solids (%):	10.4
Water to – Pump (kg/h):	8.5		
Flash Vapor (kg/h):	21.6		

Calculated Results:

Solids Solubilized (%):	72.31
Monomer/Total Sugar Ratio (%):	
Glucose:	35.46
Xylose:	45.63
Glactose:	67.96
Arabinose:	78.37
Mannose:	

Carbon Balance: Prefreatment

	Unpretreated		Pretreated											
	Dry Feed	*Carbon In*	*In Solids*			*In Liquid*				*In Flash*			*Total*	
Component	(% dry weight)	(C-mole/h)	(% dry weight)	(C-mole/h)	(% C In Feed)	(g/L mon.)	(g/L total)	(C-mole/h)	(% C In Feed)	(g/L)	(C-mole/h)	(% C In Feed)	(C-mole/h)	(% C In Feed)
Glucose	39.9	462.234	43	137,943	29.8	29.5	83.2	229.947	49.7				369.074	***79.8***
Mannose	0	0.000	0	0.000	***0.0***	**0.0**	0	0.000	0.0				0.000	
Galoctose	7.7	09.203	4.8	15.398	17.3	7.0	10.3	**26.467**	31.9				43.865	49.2
Xylose	21.4	250.232	10.8	34.646	13.8	26.1	57.2	158.089	63.2				192.735	77.0
Arobinose	12.2	141.335	3.5	11.228	7.9	30.8	***39.3***	108.617	*76.9*				119.845	04.8
Acetic Acid							3.8	10.502		0.6	0.4		10.934	
Formic Acid							0	0.000						
Lactic Acid							1.7	4.698					4.698	
Lignin	16.6	275,963	24.97	114.948	41.7		11.1	44.023	16.0				158.972	57.6
Furfural							***0***	0.000	*0.0*	**0.1**	0.1	***0.0***		
HMF							0.3	1.184	0.2					
Total	*89.2*	***1218.966***	*80.6*	*314.163*	*25.8*			***585.528***	***48.0***		***0.5***	***0.0***	***900.123***	

Ignores protein

C Recovery: **73.84%**

concentrations. A spike in glucose concentration occurred at about 180 hours because a large amount of enzyme was accidentally added to the fermenter (as discussed in section 2.3.2). The enzyme preparation contains high concentrations (300 g/L) of sucrose, which analyzes as glucose by the HPLC method.

Figures 6 and 7 presents the same type of information for the second and third 9000-L fermenters, respectively. These fermenters were less affected by the upsets that occurred in the first fermenter. The temperatures increased to 37°C and 35°C in each of these vessels during the power failure, which was probably not high enough to kill a significant fraction of the microorganisms. Component concentrations showed the same trends as the first fermenter, but the variations were less severe and can be attributed to the flow of material down the chain, rather than a direct response. Figure 8 shows that ethanol concentration increases in each of the successive ferrnenters down the continuous train.

Figure 9 shows monomeric arabinose, xylose, and galactose in the pretreated feed and the first 9000-L fermenter. The concentrations in the fermenter will always be lower than the pretreated feed because of dilution of the feed occurring in the first fermenter. Pretreated feed solids concentrations were reduced from 32% to 24%—28% in the fermenter because of water added to thin the slurry in the mixer, inoculurn, and enzyme additions. In general, the fermenter concentrations parallel the concentrations in the incoming feedstock. There was some indications of arabinose consumption, both by the drop in the fermenter arabinose Concentration beginning at about 72 hours, and in the material balance data to be presented later. It is highly unlikely that yeast will consume arabinose, particularly in an anaerobic environment. Arabinose was probably consumed by the contaminant.

Figure 10 shows the plate counts of viable yeast in each of the three fermenters, as well as the counts for the Contaminant cells in the first fermenter. During the first few days of the run, yeast cell concentrations decreased. Contaminant concentrations increased rapidly between 50 and 70 hours into the run. This occurred at the same time ethanol concentrations were high and glucose concentrations were low. Bench-scale data suggest that a 24 hours residence time is too short for adequate cell growth, and therefore, the yeast will wash out of the fermenters unless there is a continuous inoculum. Also, it is currently not possible to measure cell concentrations in SSF and viable cell counts do not measure cell concentrations. Work is needed at the bench-scale to identify a accurate method for measuring cell concentrations.

The drop in viable yeast-cell counts could be attributed to two potential causes or combination of these causes. First, less glucose is available for the yeast because of consumption by the contaminant. Also. yeast concentrations are high during initial operation of the fermenter because it was operated in batch mode with an aerated broth. This allows cell concentrations to increase. Once continuous operation begins, the cells are washed out and growth decreases as the fermenter become anaerobic. Some equilibrium level should be reached that depends on the cells added with the inoculum and cell growth.

Contamination was observed in the first 9000-L fermenter four hours after inoculation. The contaminant appeared to be a small rod under the microscope. By 24 hours, contaminant concentration was $1x10^6$ cells/mL in the second 9000-L fermenter. Contamination was observed in all three 9000-L fermenters by 48 hours. By 72 hours, the contaminant had reached a level of $1.3x10^8$ cells/mL in the first fermenter and even higher in the second and third fermenters. By 144 hours, the contaminant had decreased in first fermenter to $7.0x10^7$ cells/mL and $1.7x10^7$ cells/mL in the second fermenter. It was not possible to count the cells in the third fermenter, but the counts were decreasing in all three vessels. Although the data are limited at the end of the run, the high temperature spike and/or lowering of the pH appears to have decreased the concentration of contaminant cells. More discussion of contamination will be presented in section 4.1,

Table 6 shows material balance information (see Appendix for more detailed information) for the SSF process based on a sample taken near the end of the run (May 8, 02:00, at 212 h). This material balance was done to obtain some information on the process and is based on several assumptions that reduce the reliability of the estimate. For example, none of the process flows into the first 9000-L fermenter were constant. Several times during the run, feed was lost from the APR and inoculum and enzyme were batch additions. Therefore, average flow rates over the previous three days before the sample was taken were used in the calculations. Flow rate data for inoculum and enzyme additions were based on the decrease in level versus time data for their respective hold tanks. Another major assumption in the calculation was no loss of ethanol and water in the exhaust gas. It was also assumed that there was no additional cell mass production in the SSF fermenters from either the yeast or the contaminant. This will have little effect on the mass balance because little of the carbon is tied up in cell mass.

Table 6. SSF Material Balance

Conversions (%):	
Glucose	75.7
Galactose	46.3
Mannose	80.4
Xylose	16.1
Arabinose	38.9
Lignin	39.0
Yields (g/100 g C6 consumed)	
Ethanol	24.6
Acetic Acid	9.8
Lactic Acid	14.1

The overall carbon recovery was 74.5%, with 93% of the starch and cellulose converted to C6 sugars (total soluble sugars, including monomers and oligomers) and 71% of these sugars were converted to other products. The ethanol process yield defined as the ethanol produced divided by total potential ethanol from available C6 sugars was low at 34%. The ethanol metabolic yield is a little higher at 43% and is defined as the total ethanol produced divided by potential ethanol from consumed C6 sugars. Metabolic yield will always be higher than the process yield because of residual unconverted sugars. The production of acetic and lactic acid probably reduced the ethanol yield, and some ethanol was likely lost in the off-gas.

Table 6 shows the conversions [(in - out)/in] and major product yields. Note the 39% loss of arabinose, along with a 16% loss of xylose. The xylose loss is small and may not be significant, but the arabinose loss is significant and indicates possible consumption by the contaminant, There was also a 39% loss of lignin. This has also been seen in bench-scale data and may be a problem with the analysis of lignin in the raw corn fiber and/or fermentation broth. The problem is being investigated by the NREL analytical group.

A SSF run in shake flasks was performed on pretreated corn fiber generated during the run for comparison with SSF performance in the PDU. Average component concentrations of two duplicate flasks for 10 IU/g and 25 IU/g cellulase loadings are shown in Figure 11. Glucose (not shown) stayed between 2–3 g/L throughout the fermentation. Early data is not available because the slurry was too thick to sample. The initial solids loading was 25%, comparable to the loading in the PDU fermenters. The ethanol process yields were 38% and 41% for the 10 IU/g and 25 IU/g cellulase loadings, respectively. This compares to 34% (at 10 IU/g) achieved

in the PDU. Thus, the yields in the uncontaminated shake flask were only slightly better than the yields obtained with contaminated conditions in the PDU. This result along with the high residual glucose levels may indicate a problem with the performance of this yeast or a mass transfer limitation in the presence of high solids levels. Note that in absence of a contaminant, there is little acetic acid and no lactic acid production by this yeast. This proves that the contaminant produced the lactic acid.

4.3 Centrifuge Testing

After completion of the run, the PDU centrifuge was tested to verify operation and separation efficiency of the unit on corn fiber fermentation broth. This was the first test of the centrifuge system. The centrifuge was fed from two locations, the third 9000-L fermenter (V-455C) and centrifuge feed tank (V-601). This allowed the centrifuge to be tested with two different flow rates. This was necessary because the normal feed system originating at the centrifuge feed tank has a faulty flowmeter and a control valve that may be oversized, which did not allow control and adjustment of the flow rate. The flow control valve in this system was left in a nearly closed position and still delivered 3.3 gal/min to the centrifuge. To test a lower flow rate, the centrifuge was fed by the pump on the bottom of the fermenter. At a pump speed at 10%, the flow rate was 1.2 gal/min.

For each of the two flow rates, the centrifuge was started and run as specified in the vendor procedures. The centrifuge has one easily adjustable parameter, the back-drive speed. For each of the two flow rates, the centrifuge was run at 3 back-drive speeds; 1 (highest speed), 3, and 6.5 (lowest speed). At each backdrive speed, samples were taken from the cake (solid) stream and the centrate (liquid) stream after the centrifuge appeared to reach steady state.

All samples were analyzed for total solids by oven drying. The samples were also analyzed for apparent solids by centrifuging them in the Sorvall RT 6000D bench-top centrifuge at 5000 rpm for 10 minutes. The apparent solids are defined as the volume of the cake fraction in the centrifuge tube divided by the total volume in the centrifuge tube.

The results are shown in Table 7. As the backdrive speed is decreased, the cake becomes dryer because the solids have a longer retention time in the centrifuge. This would also be expected to increase the solids carry over into the centrate stream, however, this is not shown by the apparent solids data. The centrifuge operated well at either of the two flow rates, which is expected because the unit is designed for flow rates from 1–5 gal/min. The pond level can also be adjusted inside of the centrifuge, if the separation needs to have a clearer centrate or a dryer cake.

The current feed system (flow control valve) may not be capable of controlling the low flow rates that are normal for this plant at steady state. The system may need to be replaced with a variable speed pump that pumps directly to the centrifuge. However, the system will be checked out once the flow meter is repaired.

5.0 Other Issues

5.1 Contamination

The objective of this work was to identify the contaminant isolated from the 9000-L fermentation vessels and to identify its source. Contamination checks were performed routinely during the run and source checking and contaminant identification work was conducted after the run.

Table 7. Total and Apparent Solids in Cake and Centrate Steams

Flow Rate (galimin)	Backdrive Speed	Cake		Centrate	
		Total Solids (%)	Apparent Solids (%)	Total Solids (%)	Apparent Solids
1.2"	1	26.2	46.8	15.8	15.3
	3	30.0	63.8	20.4	12.2
	6	31.4	80.9	20.9	8.3
3.3[b]	1	30.0	74.0	15.9	15.0
	3	30.8	82.0	15.8	12.0
	6	33.7	89.4	15.8	8.7

[a] Starting material was 23.1% total solids and 35.3% apparent solids
[b] Starting material was 18.1% total solids and 23.4% apparent solids

5.1.1 Material and Methods

Contamination checks were performed on all fermentation vessels every 12 hours throughout the run. Checks were performed by inoculating solid (streak plates) and/or liquid YPD medium (1% w/v yeast extract, 2% w/v peptone, 2% w/v glucose, pH 5) with a fermentation sample and incubating at 30°C. In addition to streak plates and liquid culture checks for Contamination, cell counts of yeast and contaminants were performed roughly every 24 hours.

A study was designed and carried out to test for possible sources of Contamination. The potential sources tested were water added to the APR, CPN enzyme from the enzyme storage tank, raw corn fiber, and pretreated corn fiber. A sample from-each source was plated on three different commercial media: MRS agar (10 g/L MRS agar, pH 6.2 ± 0.2) to detect lactic acid bacteria, nutrient agar (NA, 23 g/L nutrient agar, pH 7.4 ± 0.2) to detect *Bacillus* species, and ethanol yeast-extract agar (EtOH, 20 g/L ethanol, 10 g/L yeast extract, 12 ml/L nystatin, 5 U/ml Penicillin-G, 30 g/L agar, pH 4.2) to detect acetic acid bacteria. The number and types of colonies were counted after incubating the plates at 30°C for 4 days. Table 8 summarizes the dilutions used for each sample. A sample of raw and pretreated corn fiber were also sonicated for 30 minutes before plating to disperse any clumps.

To identify the contaminant, rapid identification test kits produced by bioMérieux were employed (API 20E, API 50 CHB and API 50 CHB test kits). These kits allow identification of *Bucillzis* and *Lactobacillus* species; two of the most likely contaminants.

5.1.2 Results and Discussion

No growth was observed on plates of the water sample or pretreated corn fiber. The pretreated sample was taken during a period of steady operation of the APR, where pretreatment conditions were severe enough to sterilize the material. A sample should be taken during APR startup and tested for contamination. It might be necessary to adopt start-up procedures to prevent contamination in product that has been improperly pretreated from getting a start in the fermentation vessels. A number of microorganisms were observed (from

yeast and molds to bacteria) on all media types for the raw corn fiber samples. It was expected that raw corn fiber would be heavily contaminated. In addition, the enzyme sample had three bacterial colony types, two small rods and one large rod.

Table 8. Samples Taken and Checked for Contaminants

Sample	Dilutions Plated on MRS, NA and EtOH Plates (in duplicate)			
Fermenter Sample	0	10^1		
Water Added to APR	0	10^1		
CPN Enzyme	0	10^1		
Raw Corn Fiber	10^1	10^2	10^4	10^5
Raw Corn Fiber - Sonicated	10^1	10^2	10^3	10^4
Pretreated Corn Fiber	10^1	10^2		
Pretreated Corn Fiber - Sonicated	10^1	10^2	10^3	10^4

After the plating studies, the fermentation contaminant and the enzyme isolates were typed with the bioMérieux test luts to determine their identity. The contaminant from the fermentations was *Bucillus lentus*. Two of the organisms in the enzyme were identified as *Lactobacillus* ***buchneri*** and *Bacillus cereus*. The other one was not identified as it did not grow in the test kit. **All** of these organisms are spore formers. *Bucillus lentus* is a small, gram positive rod found in soil. It is interesting to note that the bacteria has been isolated from a potato starch production facility for the purpose of looking at its ability to produce a-amylase. Although the source is still unknown, the data suggest that the contaminant may come from corn fiber, because it is a soil bacteria with the ability to produce a-amylase and thus able to consume starch. Further testing should be done to see if *Bucillus lentus* can be isolated from the raw corn fiber. Additionally, the lime slurry will also be tested in the future.

Further testing was performed on an enzyme prep from CPN and Iogen's industrial and food grade enzyme preps to determine microbial loadings. Table 9 shows the plating results from this study. Two distinct colonies grew on the nutrient agarplates, whereas one colony type grew on the MRS plates. The results show that the food grade enzyme does not have as high a bacterial loading **as** the CPN or Iogen industrial grades. Although the enzyme preps contain significant levels of microbial contaminants, the use of unstenlized enzyme such **as** is used in the PDU has not been a problem in bench-scale fermentations. This observation, and the fact that the enzyme contaminant was not found in the 9000-L fermenters, would suggest that the enzyme prep is not likely the source of the contamination.

5.2 Review of Run Specifications

The following is a list of items defined in the run specifications, and a short discussion of how each of these specifications were satisfied.

1. Run tlie integrated pretreatment **and SSF** processes in continuous mode for 10 **days** on pretreated corn fiber using the L1400 parent strain.

Table 9. Number of Cells Counts on Different Media as a Function of Dilution

Sample	Nutrient Agar		MRS	
	0	10'	0	10'
CPN	tntc[a]	2	tctc	62
Iogen Industrial Grade	tctc	overgrown[b]	tctc	65
Iogen Food Grade	overgrown	0	23	2

[a] Too numerous to count
[b] Large number of colonies overtook plate

The pretreatment reactor (APR) and SSF fermenters were operated for 10 days. Minor shutdowns of the APR occurred throughout the run but were generally less than two hours. The SSF fernienters were fed continuously by the APR and functioned well except manual additions of inoculum and enzyme were required as well as manual level control. To become fully functional (i.e., from a mass balance perspective), the fernenters still need work on the feed addition systems, exhaust gas measuring systems, and the acid and base delivery system.

2. Utilize at least three stages (fermenters) in SSF.
This run utilized three fermenters for SSF.

3. Ferment at least 15% pretreated solids in SSF.
Total solids concentrations (based on raw feed) in the SSF fermenter ranged from 24%—28% as determined by feedstock moisture content and flow rate measurements. Mechanically operation at this solids level was satisfactory. There is some concern about the effect this level of solids had on yeast kinetics.

4. Close the carbon balance around the SSF process to ± 15%. Carbon dioxide will be calculated based on produced ethanol using stoichiometry. Ethanol in gas phase will be considered negligible.
The carbon balance around the SSF process was closed to only 75%. This was at least due in part, to the analytical problem in determination of lignin. Solving this problem would increase the carbon closure to approximately 80%—85%. A second source of error is likely liquid losses in the off-gas.

5. Complete a carbon balance calculation around the pretreatment reactor.
A carbon balance was completed on two samples taken from the APR. In general, the balances were rather poor at 74% and 76%, but could be improved by collecting flow rate measurements and improving operation of the condensers of the condensers on the flash downstream of the AFR.

6. Keep microbial contamination low as defined by less than 25% of the carbon present as glucan and xylose should be lost *to* by-products other than yeast cell mass and those by-products known to be produced by the yeast.
It was obvious from the viable cell counts that contamination was high in this run. Material balance data also shows that approximately 25% of the carbon went to lactic and acetic acid production, likely produced by the contaminant. This is unacceptable and future work must reduce contaminant concentrations and by-product formation.

7. Use mass spectrometer **(MS)** to determine component concentrations in the fermenter exhaust streams.
   The MS **was** not used **during** this **run** because of equipment problems.

8. Test centrifuge flow system on fermentation broth.
   The centrifuge flow system and centrifuge were tested with fermentation broth. There is a problem with the feed system flow meter that must be resolved before the system is operational. In event the feed system will not work, then using a rotary lobe pump to feed the centrifuge is an option.

6.0 Summary

This was the second major run of the PDU and occurred approximately four weeks after finishing the first run. Many of the problems identified **with** the 9000-L fennenters during the first run has not been corrected in time for the second run. The problems that still need to be resolved and **fixed** are automation and control of feed additions (CSL, enzyme, inoculum), automation of level control, tighter shutoff of the pressure control valves, measurement of acid/base additions rates, **and** measurement of exhaust gas flow rates. It will be necessary to resolve these problems before the next run to increase our confidence in the carbon balance information.

Contamination was a major problem for the run. **A** likely source of contamination is the cleaning and startup procedure, particularly the initial product from pretreatment before operating conditions are fully established. Future operation should divert startup material away from the reactors until steady operating conditions are reached and provide means to insure lines and equipment are sterile before feed goes through them **into** the fermenters..

7.0 Acknowledgments

The following staff members contributed to the successful operation of the plant during this **run:** Brian Boynton, John Brigham, Nancy Combs, James Dickow, Roger Duwe, Jody Farmer, James Hora, Kelly Ibsen, Ed Jennings, James Johnson, Tim Johnston, Will Keutzer, John Lesko (Amoco), Quang Nguyen, Robert O'Conner, Tim Plummer, **Dana** Rice, **Mark** Ruth, Dan Schell, Ralph Smith, Ryan Stoner, Ian Thompson, Susan Toon, and Mel Tucker. Christos Hatzis supplied the original material balance spreadsheets that were subsequently modified for use with PDU data. This report was put together with written contributions from Bob Lumpkin (Amoco), **Mark** Ruth, Dan Schell, and Susan Toon.

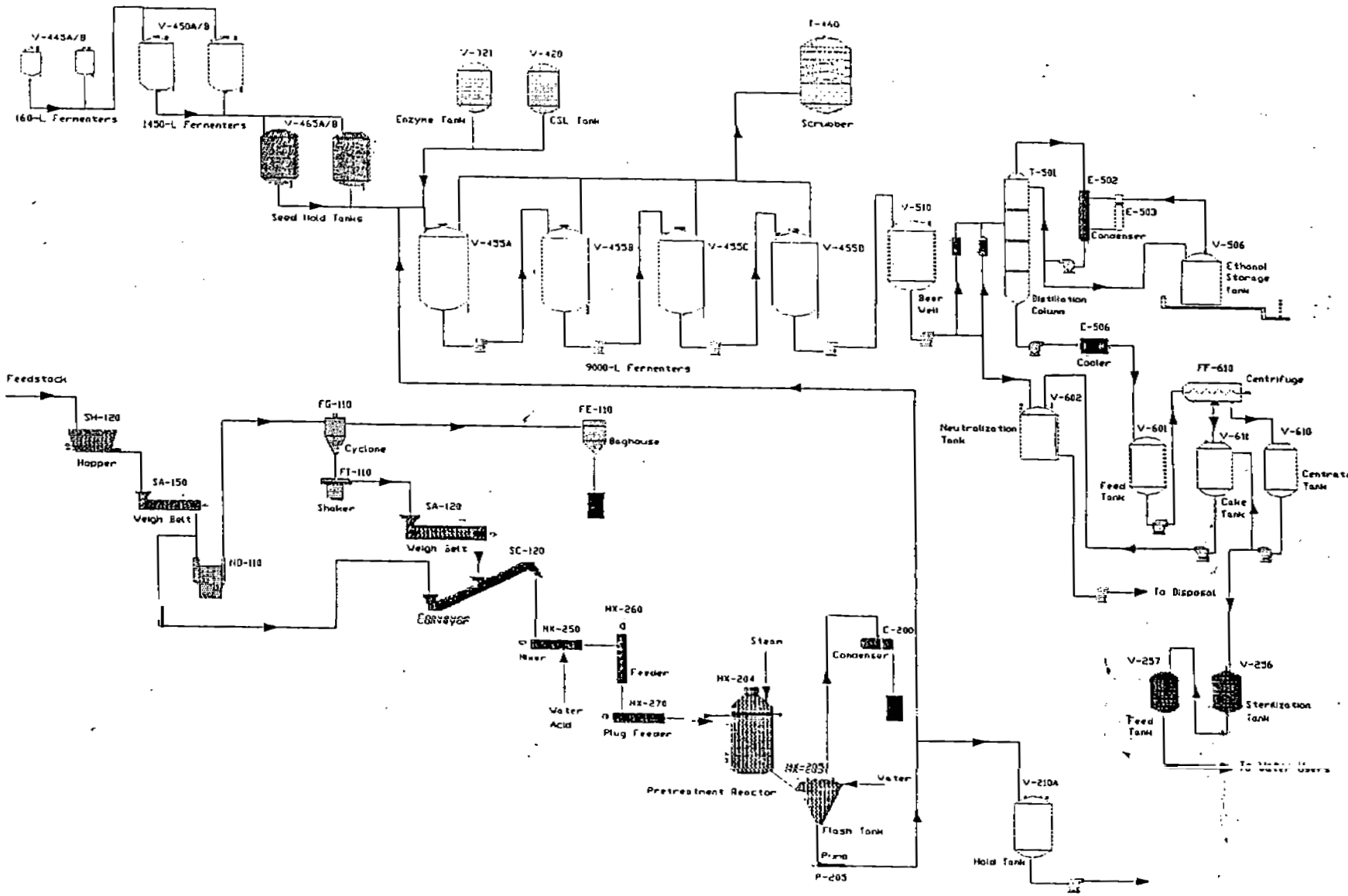


Figure 1. PDU Process Flow Diagram

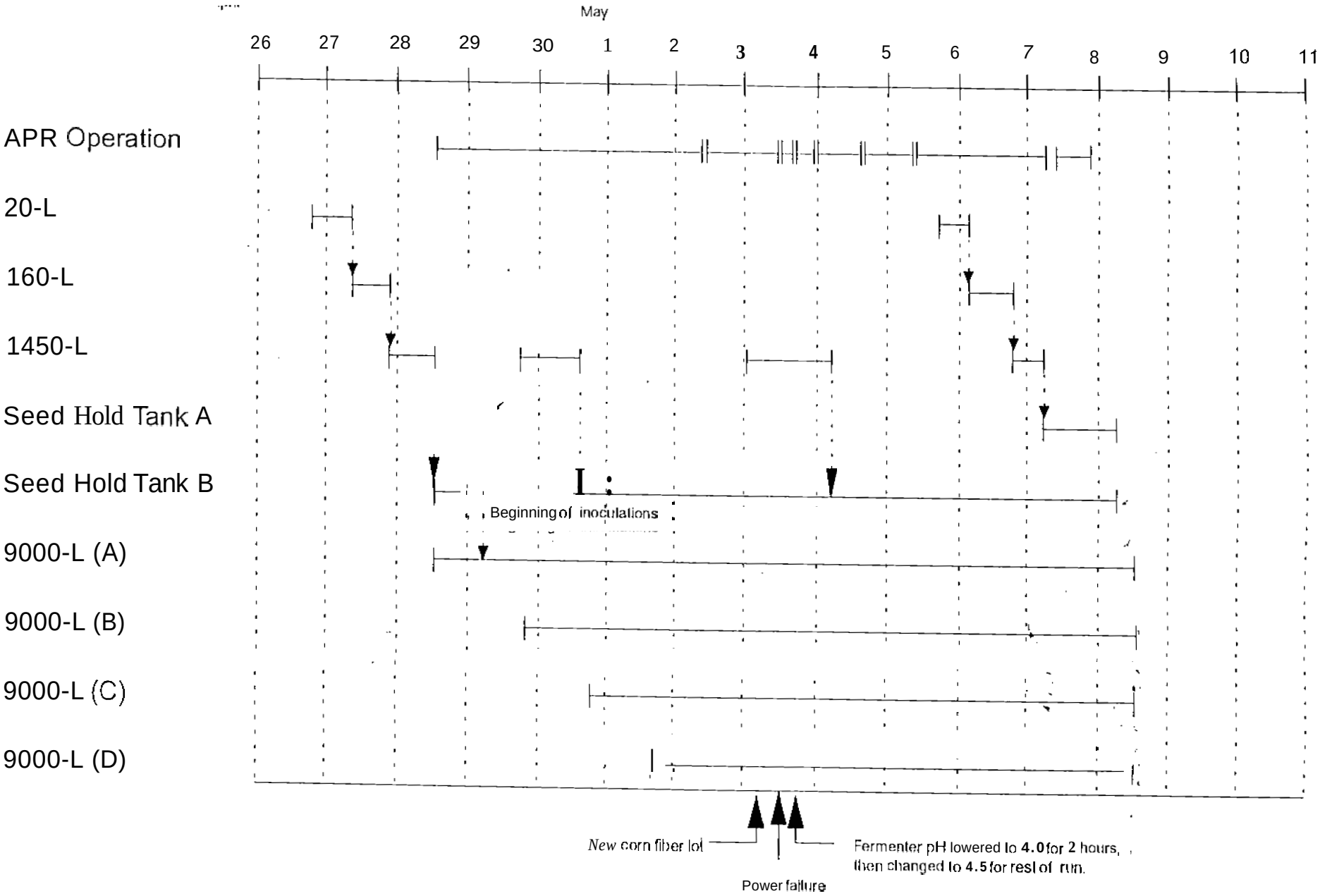


Figure 2. PDU Run History

Figure 3. Component Concentrations in APR Sample Liquor From Run P950425CF

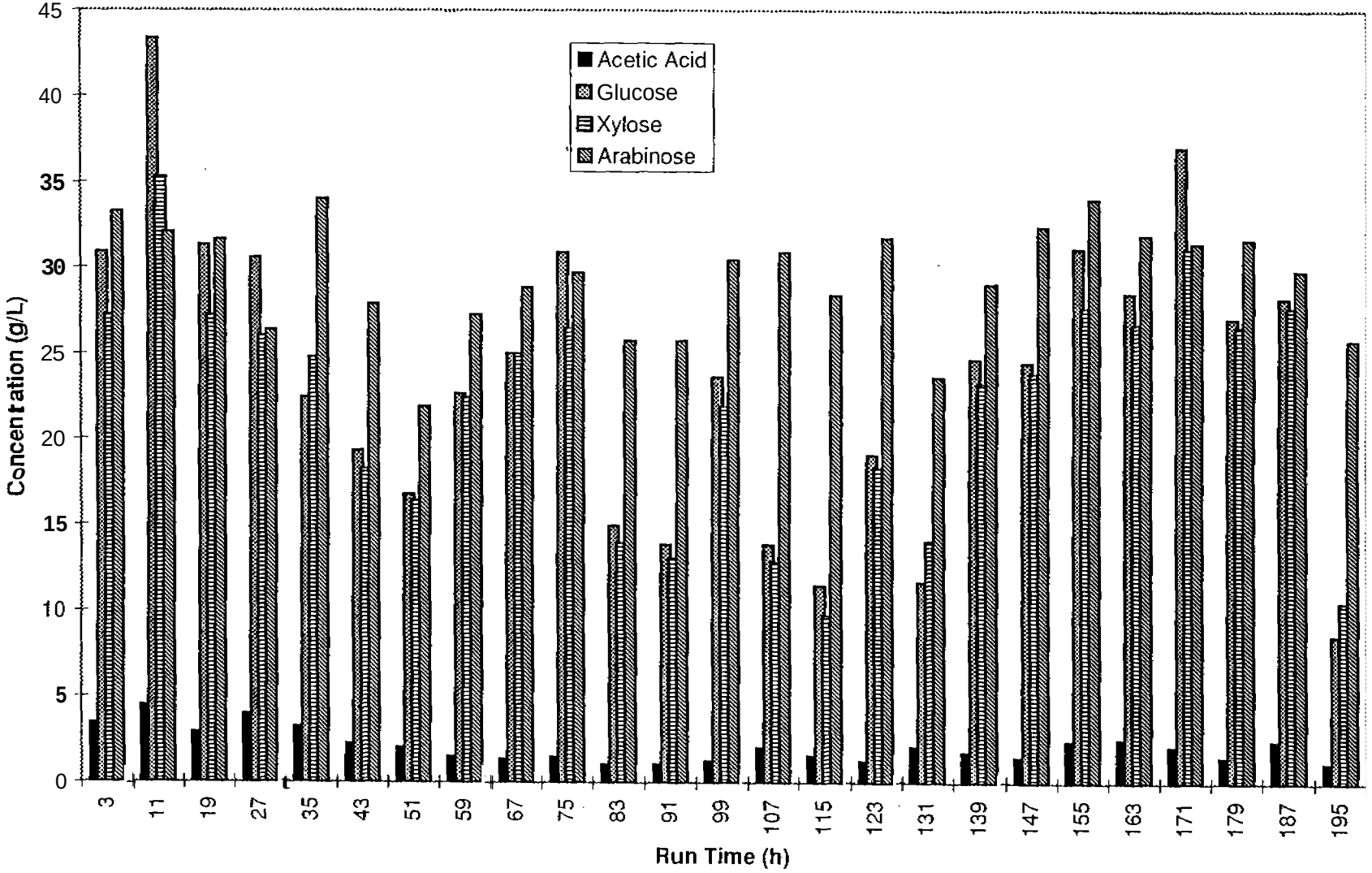


Figure 4. Monomer to Total Sugar Ratio in APR Sample Liquor From Run P950425CF

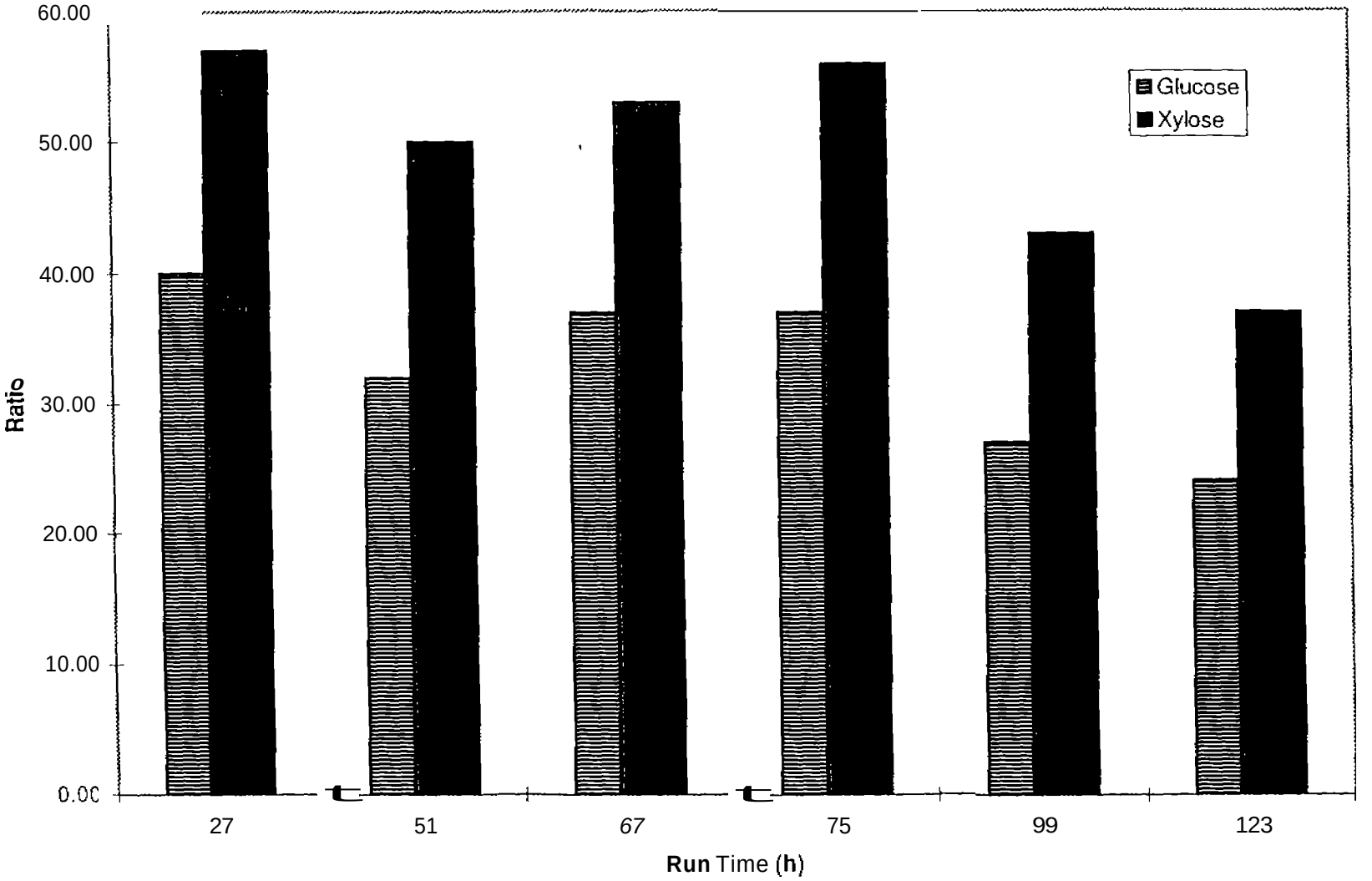


Figure 5. Component Concentations, Temperature, and pH in the First 9000-L Fermenter

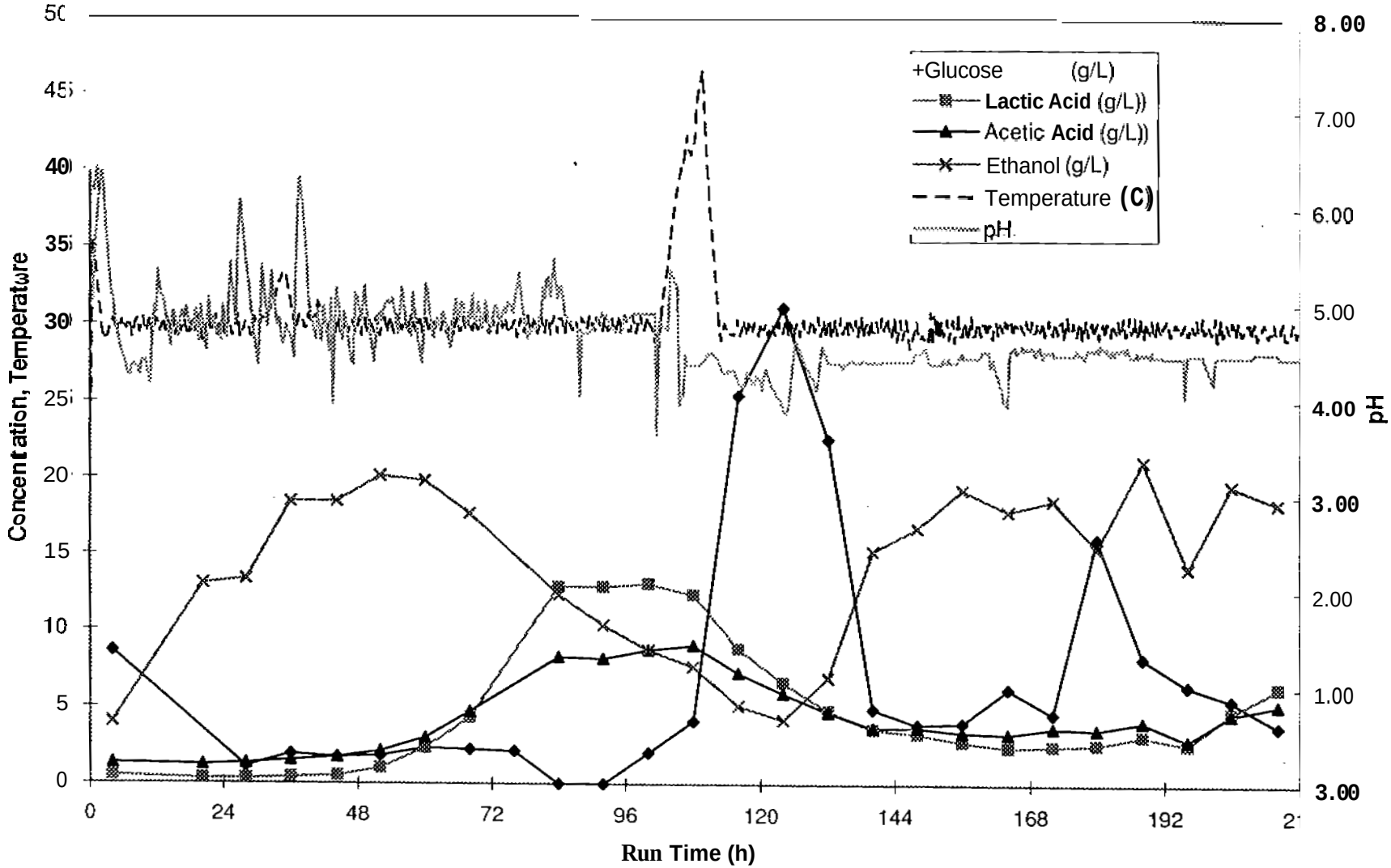


Figure 6. Component Concentrations, Temperature, and pH in the Second 9000-1 Fermenter

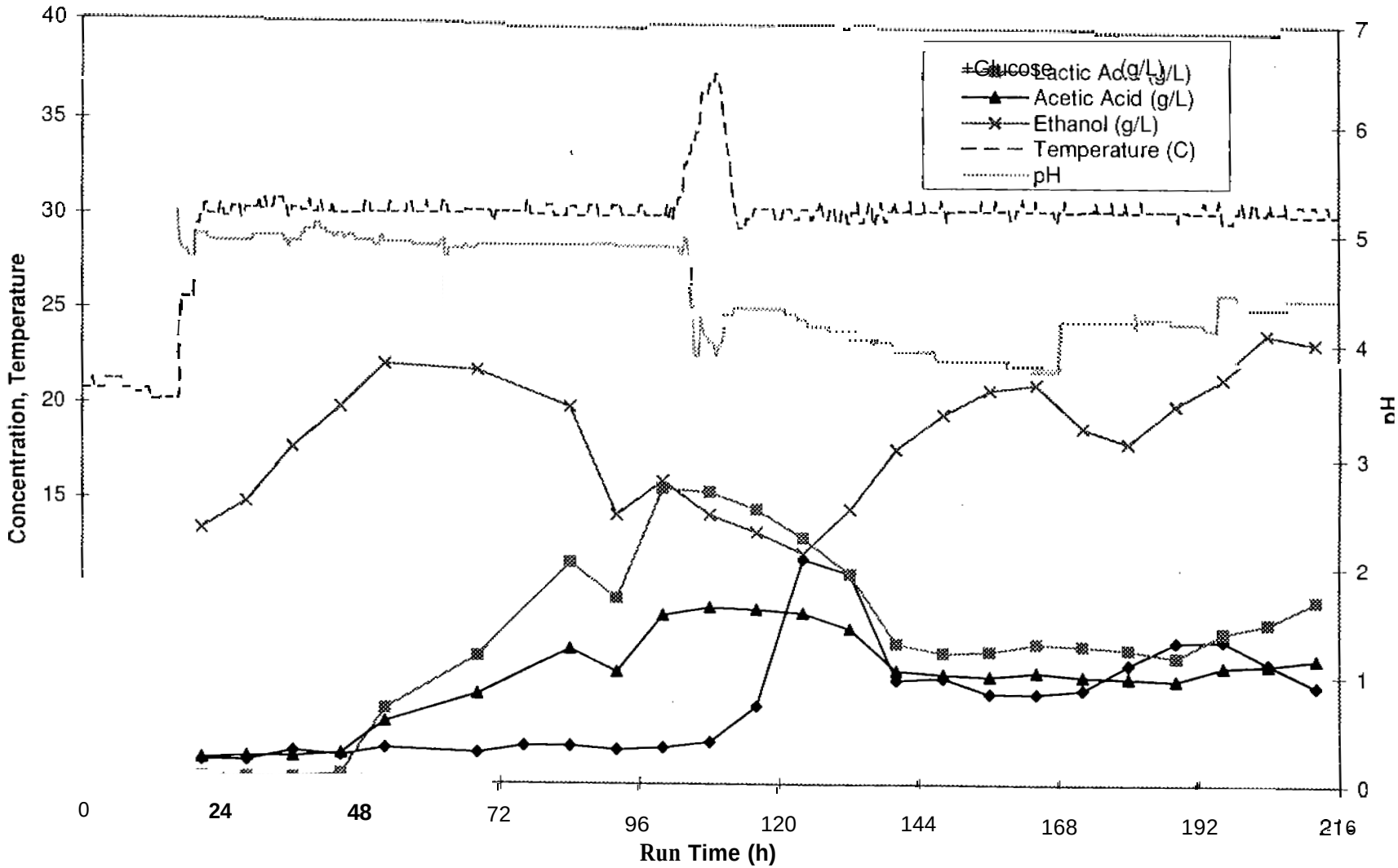


Figure 8. Ethanol Concentrations in the 9000-L Fermenters

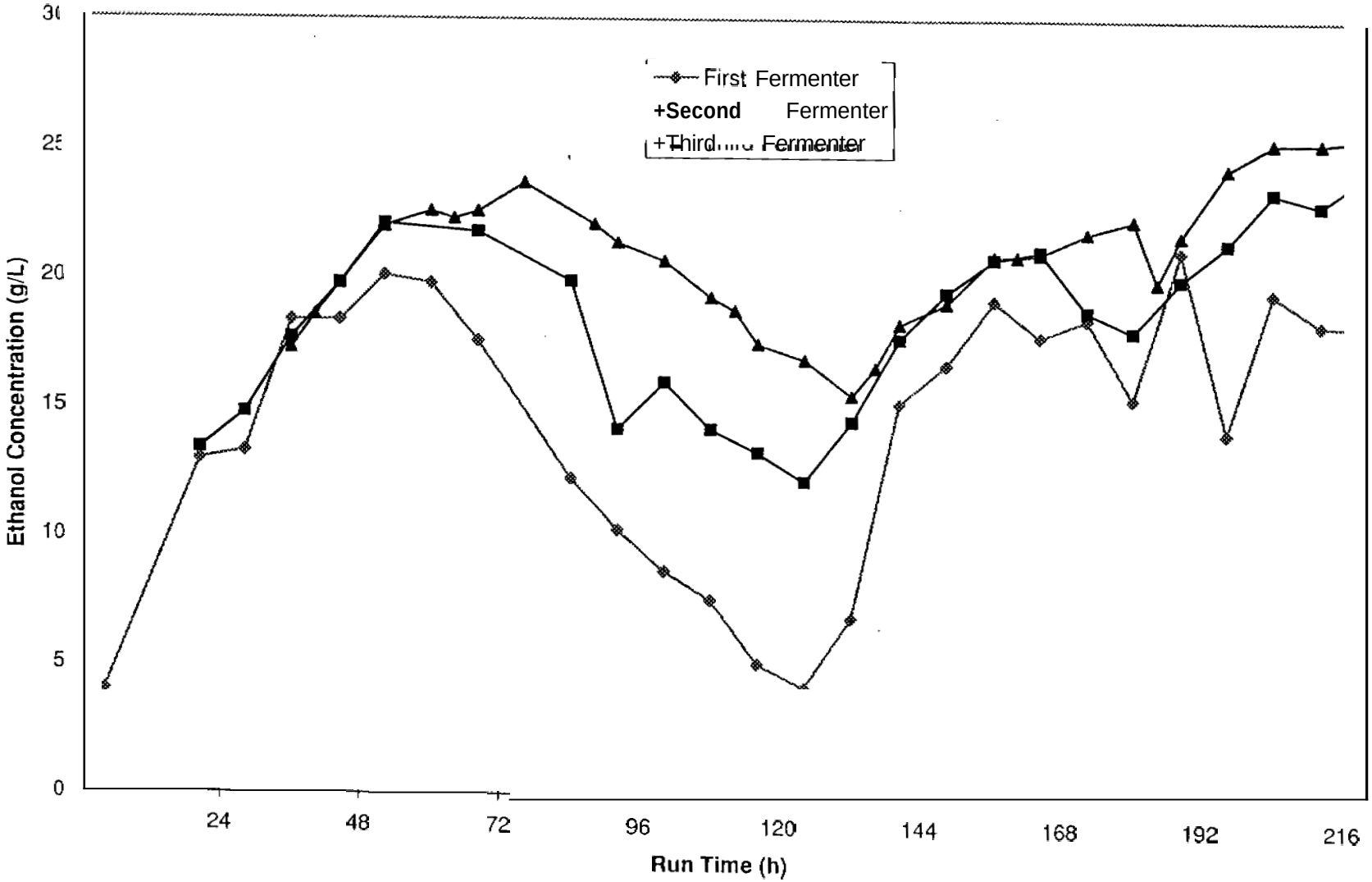


Figure 7. Component Concentrations, Temperature, and pH in the Third 9000-L Fermenter

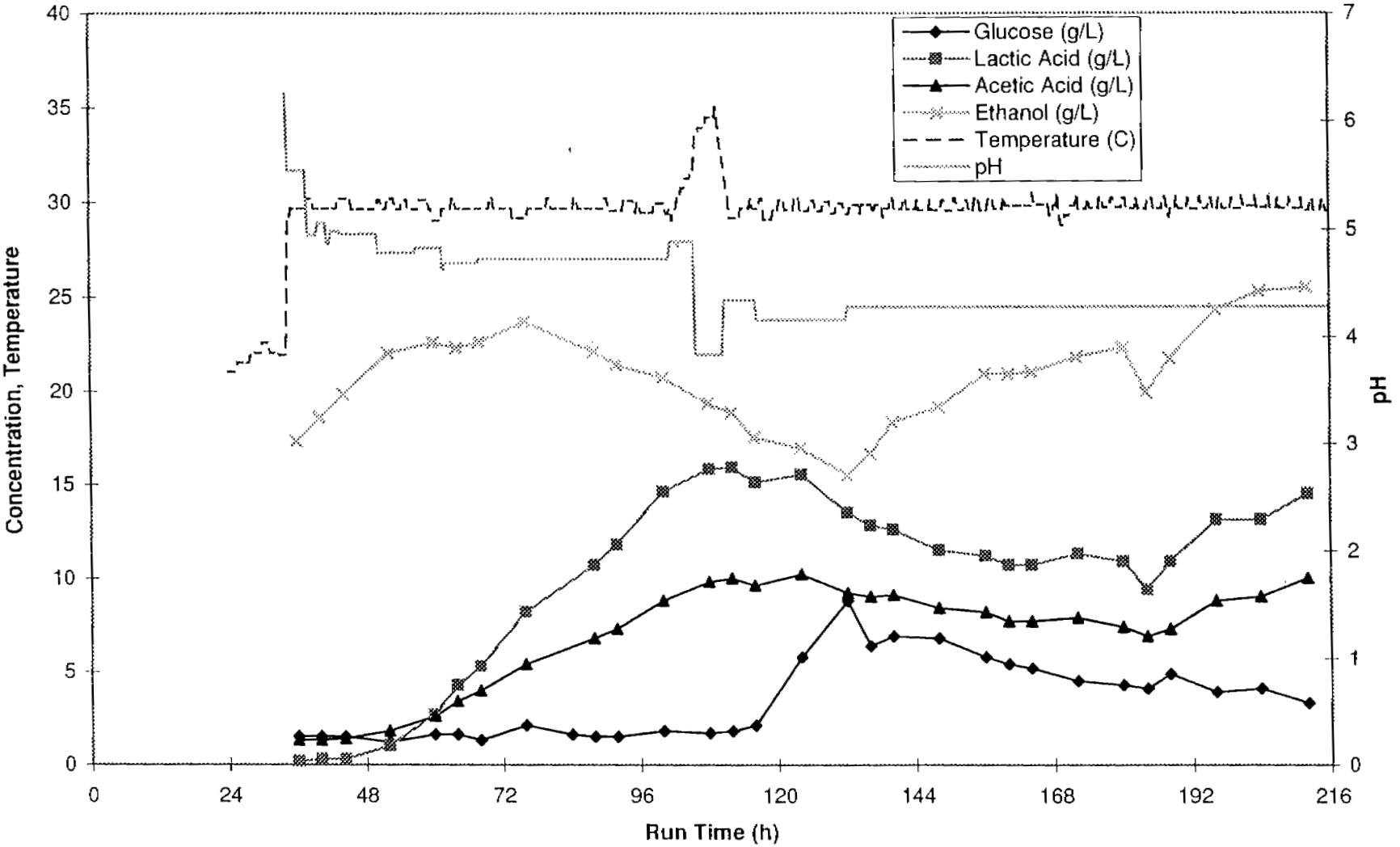


Figure 9. Monomeric Sugar Concentrations *in* Pretreated Feed and the First 9000-L Fermenter

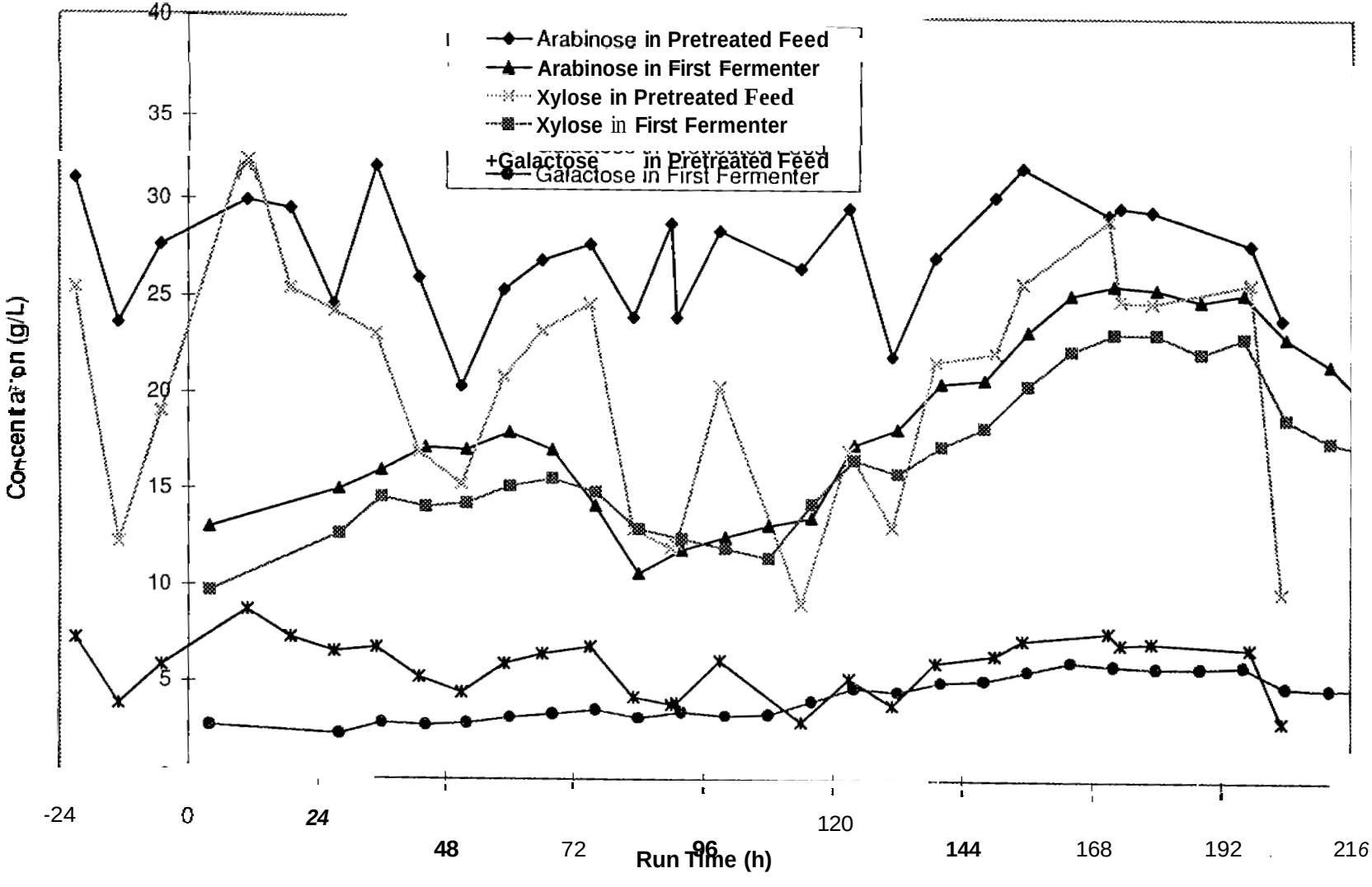


Figure 10. Viable Yeast and Contaminant Counts in the 9000-L Fermenters

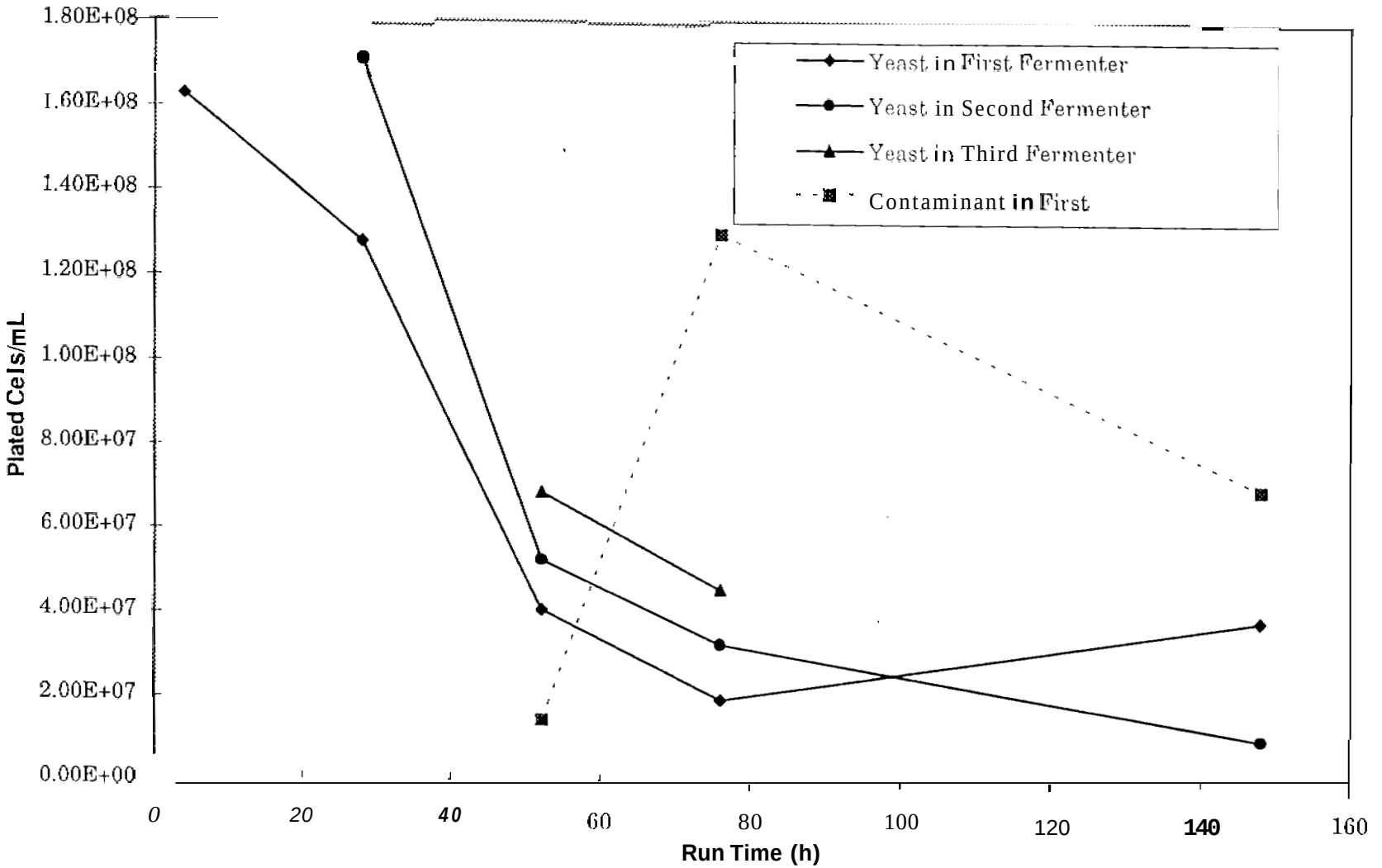
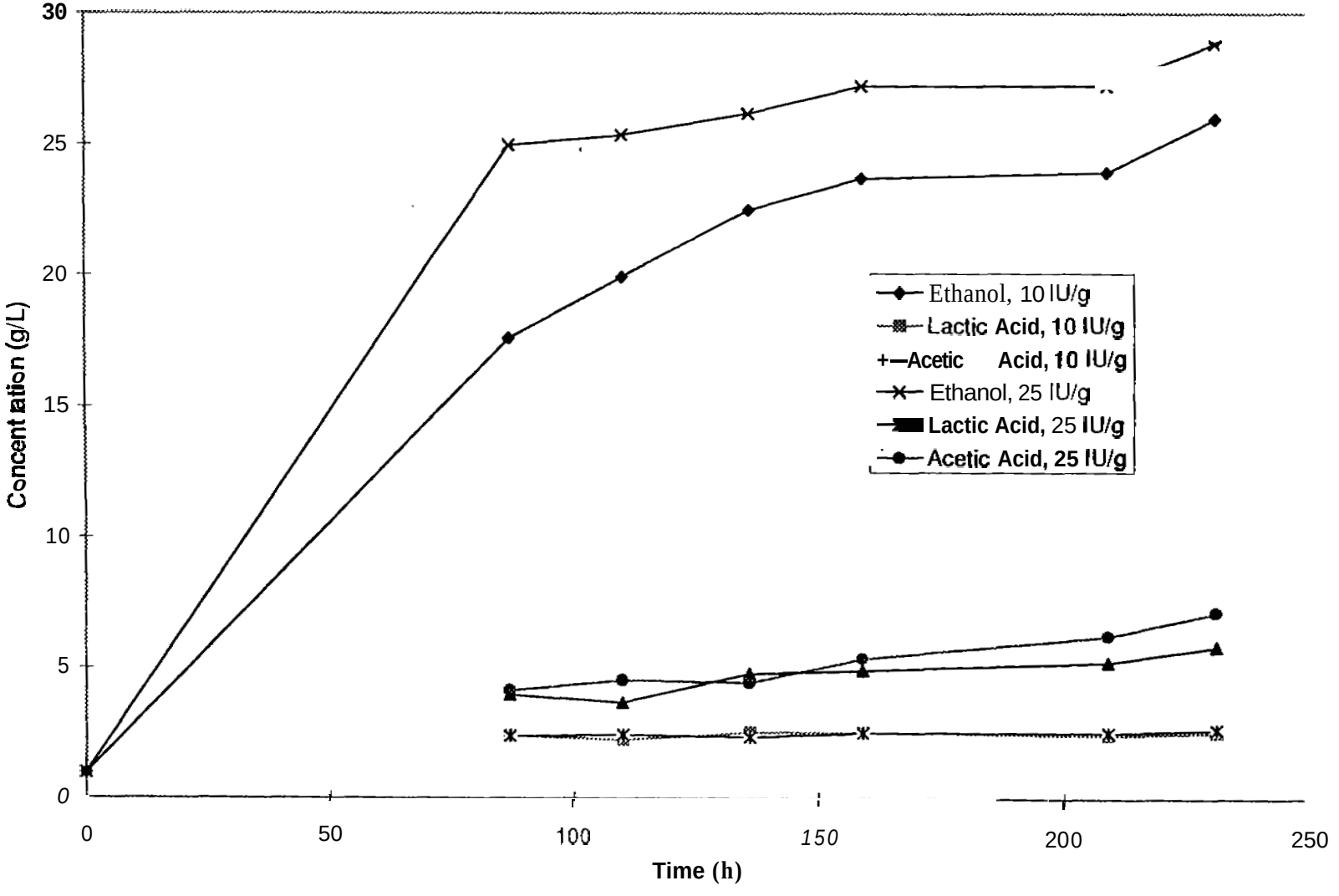


Figure 11. Concentrations in Shake Flask SSF of Pretreated Corn Fiber



APR Sample Data

Sample #	Date	Time	Run Time (h)	Monomers (acid column)						
				Glucose (g/L)	Xylose (g/L)	Arabinose (g/L)	Lactic (g/L)	Acetic (g/L)	Furfural (g/L)	HMF (g/L)
APR-038	29-Apr	9:00	3				1.5022	3.4336	0.1073	0
APR-039	29-Apr	17:00	11				1.3949	4.5066	0.2146	0
APR-040	30-Apr	1:00	19				1.2876	2.8971	0.1073	0
APR-041	30-Apr	9:00	27				1.5022	3.9701	0	0.3219
APR-042	30-Apr	17:00	35				1.3949	3.219	0.1073	0
APR-043	1-May	1:00	43				0.9657	2.2533	0	0
APR-044	1-May	9:00	51				0.8584	2.0387	0	0
APR-045	1-May	17:00	59				0.6438	1.5022	0	0
APR-046	2-May	1:00	67				0.6438	1.3949	0	0
APR-047	2-May	9:00	75				0.8584	1.5022	0	0
APR-048	2-May	17:00	83				0.8584	1.073	0	0
APR-049	3-May	11:00	91				0.7511	1.073	0	0
APR-050	3-May	9:00	99				0.7511	1.2876	0	0
APR-051	3-May	17:00	107				0.8584	2.0387	0	0
APR-052	4-May	1:00	115				0.9657	1.6095	0	0
APR-053	4-May	9:00	123				1.073	1.2876	0	0
APR-054	4-May	17:00	131				0.8584	2.146	0	0
APR-055	5-May	1:00	139				1.1803	1.6241	0	0
APR-056	5-May	9:00	147				1.2876	1.5022	0	0
APR-057	5-May	17:00	155				1.2876	2.4679	0	0
APR-058	6-May	1:00	163				1.073	2.5752	0	0
APR-059	6-May	9:00	171				1.1803	2.146	0	0
APR-060	6-May	17:00	179				1.073	1.5022	0	0
APR-061	7-May	1:00	187				1.073	2.4679	0	0
APR-062	7-May	17:00	195				1.1803	1.1803	0	0

APR Sample Data

Sample Analysis Data

Sample#	Data	Time	Run Time (h)	Monomers (lead column) Glucose (g/L)	Xylose (g/L)	Galactose (g/L)	Arabinose (g/L)	Manose (g/L)		Total Sugars (lead column) Glucose (g/L)	Xylose (g/L)	Galactose (g/L)	Arabinose (g/L)	Manose (g/L)	Total Solids (%)
APR-038	**29-Apr**	9:oo	3	30.902	27.2542	7.7256	33.263	**0**	**0**						43.6
APR-039	29-Apr	17:00	11	43.349	35.3017	9.3351	32.0827	**0**	0						30.2
APR-040	**30-Apr**	1:00	19	31.332	27.2542	7.8329	31.6535	**0**	0						**30.5**
APR-04 1	30-Apr	9:oo	27	30.581	26.0739	7.0018	26.3958	**0**	0	75539	45603	100862	3219	0	32.6
APR-042	30-Apr	17:00	35	22.426	24.7863	7.2964	34.0141	0	0						32.5
APR-043	1-May	1:00	43	19.314	18.241	5.6869	427.898	**0**	0						30
APR-044	1-May	9:oo	51	16.739	16.4169	4.8285	21.8892	**0**	0	52577	32941	78329	248936	0	31.2
APR-045	1-May	17:00	59	22.64	22.4257	6.438	27.2542	0	0						31.8
APA-046	2-May	1:00	67	25.001	25.0009	6.9745	28.8637	**0**	0	67921	47 105	1073	344433	0	31
APR-047	2-May	9:oo	75	30.902	26.5031	7.4037	29.7221	0	0	82836	47641	108373	349798	0	30.2
APR-048	2-May	17:00	83	14.915	13.949	4.6139	25.752	0	0						31.3
APA-049	3-May	11:00	91	13.842	13.0906	4.292	25.752	**0**	0						32.7
APR-050	3-May	9:oo	99	23.606	21.8892	6.6526	30.4732	**0**	0	87 128	50431	113738	387353	0	33.5
APR-051	3-May	17:00	107	13.842	12.676	4.1847	30.9024	**0**	0						33.2
APR-052	4-May	1:00	115	11.481	9.7643	3.219	28.4345	**0**	0						30.7
APR-053	4-May	9:00	123	19.099	18.3483	5.6869	31.7608	**0**	0	78651	49895	104081	358382	0	32
APR-054	4-May	17:00	131	11.696	14.0563	4.1847	23 606	**0**	0						32.7
APR-055	5-May	1:00	139	24.679	23.2641	6.5453	29.0783	**0**	0						30.2
APR-056	5-May	9:00	147	24.464	23.8206	6.9745	32.4046	**0**	0						30.1
APR-057	5-May	17:00	155	31.117	27.6834	7.8329	34.0141	0	**0**						29.3
APR-058	6-May	1:00	163	28.542	26.7177	7.6183	31.8681	**0**	**0**						30.9
APR-059	6-May	9:00	171	37.019	31.117	8.2621	31.4389	**0**	0						29.9
APR-060	6-May	17:00	**I79**	27.04	26.6104	7.7256	31.6535	**0**	**0**						30
APR-061	7-May	1:00	187	28.22	27.6834	7.4037	29.8294	**0**	**0**						29.4
APR-062	7-May	17:00	195	0.584	10.5154	3.3263	25.752	**0**	a						30.2

APR Sample Data

				(Adjusted to 21.48% solids)							Comparison to previous material					
				Adjusted Total Sugars (lead column)					Monomer/Total Sugar Ratio		Previous Total Sugars and Inhibitors (lead column) at 21.48					
Sample #	Date	Time	Run Time (h)	Glucose (g/L)	Xylose (g/L)	Galactose (g/L)	Arabinose (g/L)	Manose (g/L)	Glucose	Xylose	Glucose (g/L)	Xylose (g/L)	Galactose (g/L)	Arabinose (g/L)	Manose (g/L)	Furfural (g/L)
APR-038	29-Apr	9:oo	3													
APA-039	29-Apr	17:00	11													
APR-040	30-Apr	1:00	19													
APR-041	30-Apr	9:00	27	49.77	**30.05**	**6.65**	21.21	*0.00*	**0.40**	0.57						
APR-042	30-Apr	17:00	35													
APR-043	1-May	1:00	43													
APR-044	1-May	9:00	51	36.20	22.68	5.39	17.14	0.00	0.32	0.50						
APR-045	I-May	17:00	59													
APR-046	2-May	1:00	67	47.06	**32.64**	7.43	23.87	0.00	0.37	0.53						
APR-047	2-May	9:00	75	58.92	33.89	7.71	**24.88**	0.00	0.37	0.56						
APA-048	2-May	17:00	***83***													
APR-049	3-May	1i:00	91													
APR-050	3-May	9:oo	99	55.87	32.34	7.29	**24.84**	0.00	0.27	**0.43**						
APR-051	3-May	17:00	107													
APR-052	4-May	1:00	115													
APR-053	**4-May**	9:oo	123	52.79	33.49	**6.99**	24.06	0.00	0.24	0.37						
APA-054	4-May	17:00	131													
APR-055	5-May	1:00	139													
APR-056	5-May	9:oo	147													
APR-057	5-May	17:00	155													
APR-058	6-May	I:00	163													
APR-059	6-May	9:00	171													
APR-060	6-May	17:00	179													
APR-061	7-May	1:00	187													
APR-062	7-May	17:00	195													

POU SSF Material Balance (based on raw feed)

Run #: P950425CF
Date: 5/8/95
Time: 2:00

Run Conditions: Pretreatment Temp (C): 215 Flash Tank Temp (C) Va

Pretreatment Acid Concentration (%): 1.77 (In Liquid Phase)

Input Data:

Feed Flow Rate (kg/h):	66.0	Feed Solids Concentration (%):	45	Inoculum Flow Rate (FE 455A-4) (kg/h):	7.4
Make Water (kg/h):	7.3	98% Acid Concentration (%):	7.4	Enzyme Flow Rate (FE 455A-5) (kg/h):	3.3
Acid Flow Rate (kg/h):	19.3	Lime Concentration (%):	0	CSL Flow Rate (FE-455A-6) (kg/h):	0
Steam to APR (kg/h):	17.6			Other Additions (kg/h):	0
		Hydrolysate Insoluble Solids (%):	0		
Water to Dosing (kg/h):	8.5	Fermenter Insoluble Solids (%):	5.3	Fermentation Solids Conc. (%):	27.1
Flash Vapor (kg/h):	19.7				

Cellulose Conversion:	93.2%
Xylan Conversion:	88.5%
Overall C6-Sugar Conversion:	71.3%
Overall C5-Sugar Conversion:	24.4%
Ethanol Process Yield (% theor):	34.2%
Ethanol Metabolic Yield (% theor):	48.0%

Carbon Balance: SSF

	Carbon In												**Carbon Out**							**Conversion**	**Yield**
	In Feedstock																				
	In Raw Feed			*In Feed Liquor*			*In Inoculum*			*In Enzyme*			*Total*	*In Solids*			*In Liquor*			*Total*	*(In-Out)/In*	*g product/*
Component	(% dry wt)	(C-moles/h)	(% Total In)	(g/L)	(C-moles/h)	(% Total In)	(g/L)	(C-moles/h)	(% Total In)	(g/L)	(C-moles/h)	(% Total In)	(C-moles/h)	(% dry wt)	(C-moles/h)	(% Total Out)	(g/L)	(C-moles/h)	(% Total Out)	(C-moles/h)	(%)	100 g C6 con
Cellobiose							*0.0*	0.000	*100.0*				0.000				0.00	0.000		0.000		
Glucose	39.9	394.663	*93.3*				0.0	0.000	*0.0*	300.0	28.180	*6.7*	422.843	13.90	26.919	*26.2*	21.90	75.782	*73.8*	102.701	75.71	
Galactose	7.4	75.174	*100.0*				0.0	0.000	0.0				75.174	6.00	11.620	288	8.30	28.721	*71.2*	40.341	46.34	
Mannose	0.00	0.010	*100.0*				*0.0*	0.000	0.0				0.010	0.00	0.002	*100.0*	o.m	0.000	*0.0*	0.002	80.42	
Xylose	21.5	212.663	*100.0*				0.0	0.000	00				212.663	12.60	24.402	f3.7	44.50	153.986	*86.3*	178.367	16.12	
Arabinose	12.2	120.674	*100.0*				0.0	0.000	00				120.674	4.10	7.940	10.8	19.00	65.747	*89.2*	73.687	30.94	
Lignin	16.6	235.622	*100.0*						*0.0*				235.622	35.60	98.935	68.9	9.00	44.691	*31.1*	143.624	39.04	
Ethanol							4.300	1.381	*100.0*				1.381				25.50	115.022		115.022		24.56
Cell Mass							3.300	0.975	*100.0*				0.975				0.33	1.368		1.368		0.09
Carbon Dioxide																		56.820		56.820		23.46
Glycerol							0.000	0.000	*100.0*				0.000				0.00	0.000		0.000		0.00
Acetic Acid							0.000	0.000	*100.0*				0.000				10.03	34.604		34.604		9.75
Lactic Acid							0.940	0.232	*100.0*				0.232				1450	50.175		50.175		Id 07
Succinic Acid							0.000	0.000	*100.0*				0.000				o.m	0.000		0.000		0.00
Total	*97.807*	1038.805	*97.1*		0.000	*0.0*							*1069.573*	*72.20*	*169.818*	*21.3*		*A96.015*	*78.7*	*704.719*		*71.02*

C-RECOVERY: **74.49%**

Date	Time	Date/Time Serial number	Run time (h)	455A Glucose (g/L)	455A Lactic (g/L)	455A Acetic (g/L)	455A EtOH (g/L)
4/29/95	10:00	34818.4167	4.00	8.6	0.5	1.3	4
4/30/95	2:15	34819.0938	20.25		0.3	1.2	13
4/30/95	10:00	34819.4167	28.00	1.1	0.3	1.3	13.3
4/30/95	18:00	34819.7500	36.00	1.9	0.4	1.5	18.4
5/1/95	2:15	34820.0938	44.25	1.7	0.5	1.7	18.4
5/1/95	10:00	34820.4167	52.00	1.8	1	2.1	20.1
5/1/95	18:00	34820.7500	60.00	2.3	2.3	3	19.8
5/2/95	2:00	34821.0833	68.00	2.2	4.3	4.7	17.6
5/2/95	10:00	34821.4167	76.00	2.1			
5/2/95	18:00	34821.7500	84.00	0	12.8	8.2	12.3
5/3/95	2:00	34822.0833	92.00	0	12.8	8.1	10.3
5/3/95	10:00	34822.4167	100.00	2	13	8.7	8.7
5/3/95	18:00	34822.7500	108.00	4.1	12.3	9	7.6
5/4/95	2:00	34823.0833	116.00	25.4	*8.8*	7.2	5.1
5/4/95	10:00	34823.4167	124.00	31.1	6.6	5.9	4.2
5/4/95	18:00	34823.7500	132.00	22.5	4.8	4.7	6.9
5/5/95	2:00	34824.0833	140.00	4.9	3.6	3.7	15.2
5/5/95	10:00	34824.4167	148.00	3.9	3.3	3.7	16.7
5/5/95	18:00	34824.7500	156.00	4	2.8	3.4	19.2
5/6/95	2:00	34825.0833	164.00	6.2	2.4	3.3	17.8
5/6/95	10:00	34825.4167	172.00	4.6	2.5	3.7	18.5
5/6/95	17:50	34825.7431	179.83	16	2.6	3.6	15.4
5/7/95	2:00	34826.0833	188.00	8.2	3.2	4.1	21.1
5/7/95	10:00	34826.4167	196.00	6.4	2.6	2.9	14.1
5/7/95	17:45	34826.7396	203.75	5.5	4.7	4.6	19.5
5/8/95	2:00	34822.0833	212.00	3.8	6.3	5.2	18.3

Date	lime	Date/Time Serial number	Run time (h)	455B Glucose (g/L)	455B Lactic (g/L)	4550 Acetic (g/L)	4556 EtOH (g/L)
4/30/95	10:00	34819.4167	28.00	1.1	0.2	1.3	14.8
4/30/95	18:00	34819.7500	36.00	1.6	0.2	1.3	17.7
5/1/95	2:15	34820.0938	44.25	1.4	0.4	1.5	19.8
5/1/95	10:00	34820.4167	52.00	1.8	3.9	3.2	22.1
5/2/95	2:00	34821.0833	68.00	1.6	6.7	4.7	21.8
5/2/95	10:00	34821.4167	76.00	2			
5/2/95	18:00	34821.7500	84.00	2	11.7	7.1	19.9
5/3/95	2:00	34822.0833	92.00	1.8	9.8	5.9	14.2
5/3/95	10:00	34822.4167	100.00	1.9	15.6	8.9	16
5/3/95	18:00	34822.7500	108.00	2.2	15.4	9.3	14.2
5/4/95	2:00	34823.0833	116.00	4.1	14.5	9.2	13.3
5/4/95	10:00	34823.4167	124.00	11.9	13	9	12.2
5/4/95	18:00	34823.7500	132.00	11.1	11.1	8.2	14.5
5/5/95	2:00	34824.0833	140.00	5.5	7.4	6	17.7
5/5/95	10:00	34824.4167	148.00	5.6	6.9	5.8	19.5
5/5/95	18:00	34824.7500	156.00	4.8	7	5.7	20.8
5/6/95	2:00	34825.0833	164.00	4.8	7.4	5.9	21.1
5/6/95	10:00	34825.4167	172.00	5	7.3	5.7	18.8
5/6/95	17:50	34825.7431	179.83	6.3	7.1	5.6	18
5/7/95	2:00	34826.0833	188.00	7.5	6.7	5.5	20
5/7/95	10:00	34826.4167	196.00	7.6	8	6.2	21.4
5/7/95	17:45	34826.7396	203.75	6.4	8.5	6.3	23.4
5/8/95	2:00	34827.0833	212.00	5.2	9.7	6.6	22.9

Date	Time	Date/Time Serial number	Run time (h)	455C Glucose (g/L)	455C Lactic (g/L)	455C Acetic (g/L)	455C EtOH (g/L)
4/30/95	18:00	34819.7500	36.00	1.5	0.2	1.3	17.3
4/30/95	22:00	34819.9167	40.00	1.5	0.3	1.3	18.6
5/1/95	2:15	34820.0938	44.25	1.5	0.3	1.4	19.8
5/1/95	10:00	34820.4167	52.00	1.2	1	1.8	22
5/1/95	18:00	34820.7500	60.00	1.6	2.7	2.6	22.6
5/1/95	22:00	34820.9167	64.00	1.6	4.3	3.4	22.3
5/2/95	2:00	34821.0833	68.00	1.3	5.3	4	22.6
5/2/95	10:00	34821.4167	76.00	2.1	8.2	5.4	23.7
5/2/95	18:00	34821.7500	84.00	1.6			
5/2/95	22:00	34821.9167	88.00	1.5	10.7	6.8	22.1
5/3/95	2:00	34822.0833	92.00	1.5	11.8	7.3	21.4
5/3/95	10:00	34822.4167	100.00	1.8	14.6	8.8	20.7
5/3/95	18:00	34822.7500	108.00	1.7	15.8	9.8	19.3
5/3/95	22:00	34822.9167	112.00	1.8	15.9	10	18.8
5/4/95	2:00	34823.0833	116.00	2.1	15.1	9.6	17.5
5/4/95	10:00	34823.4167	124.00	5.8	15.5	10.2	16.9
5/4/95	18:00	34823.7500	132.00	8.8	13.5	9.2	15.5
5/4/95	22:00	34823.9167	136.00	6.4	12.8	9	16.6
5/5/95	2:00	34824.0833	140.00	6.9	12.6	9.1	18.3
5/5/95	10:00	34824.4167	148.00	**6.8**	11.5	8.4	19.1
5/5/95	18:00	34824.7500	156.00	5.8	11.2	8.2	20.9
5/5/95	22:00	34824.9167	160.00	5.4	10.7	7.7	20.9
5/6/95	2:00	34825.0833	164.00	5.2	10.7	7.7	21
5/6/95	10:00	34825.4167	172.00	4.5	11.3	7.9	21.8
5/6/95	17:50	34825.7431	179.83	4.3	10.9	7.4	22.3
5/6/95	22:00	34825.9167	184.00	4.1	9.4	6.9	19.9
5/7/95	2:00	34826.0833	188.00	4.9	10.9	7.3	21.7
5/7/95	10:00	34826.4167	196.00	3.9	13.1	8.8	24.3
5/7/95	17:45	34826.7396	203.75	4.1	13.1	9	25.3
5/8/95	2:00	34827.0833	212.00	3.3	14.5	10	25.5

V-455 Compositional Data **(Other sugars)**

(Figure 9

				Xylose (g/L)	Galactose (g/L)	Arabinose (g/L)
4/29/95	10:00	34818.4167	4.00	9.7	2.7	13
4/30/95	10:00	34819.4167	28.00	12.7	2.3	15
4130195	18:00	34819.7500	36.00	14.6	2.9	16
5/1/95	2:15	34820.0938	44.25	14.1	2.8	17.2
5/1/95	10:00	34820.4167	52.00	14.3	2.9	17.1
5t1/95	18:00	34820.7500	60.00	15.2	3.2	18
5/2/95	2:00	34821.0833	68.00	15.6	3.4	17.1
5/2/95	10:00	34821.4167	76.00	14.9	3.6	14.2
5/2/95	18:00	34821.7500	84.00	13	3.2	10.7
5/3/95	2:00	34822.0833	92.00	12.5	3.5	11.9
5/3/95	10:00	34822.4167	100.00	12	3.3	12.6
5/3/95	18:00	34822.7500	108.00	11.5	**3.4**	13.2
5/4/95	2:00	34823.0833	116.00	14.3	**4.1**	13.6
5/4/95	10:00	34823.4167	124.00	16.6	**4.8**	17.4
5/4/95	18:00	34823.7500	132.00	15.9	4.6	18.2
5/5/95	2:00	34824.0833	140.00	17.3	5.1	20.6
5/5/95	10:00	34824.4167	148.00	18.3	5.2	20.8
5/5/95	18:00	34824.7500	156.00	20.5	5.7	23.3
5/6/95	10:00	34825.4167	172.00	23.2	6	25.7
5/6/95	17:50	34825.7431	179.83	23.2	5.9	25.5
5/7/95	2:00	34826.0833	188.00	22.2	5.9	24.9
5/7/95	10:00	34826.4167	196.00	23	6	25.3
5/7/95	17:45	34826.7396	203.75	18.8	4.9	23
5/8/95	2:00	34827.0833	212.00	17.6	**4.8**	21.6
5/8/95	10:00	34827.4167	220.00	17.1	4.9	19.3

Cell and Contaminanat Concentrations
Figure (10)

Time	455A cells/mL	455B cells/mL	455C cells/mL	455A contam/mL	455B contam/mL	455C contam/ml
4	1.63E+08					
28	1.28E+08	1.71E+08				
52	4.10E+07	5.30E+07	6.90E+07	1.50E+07		4.30E+07
76	2.00E+07	3.30E+07	4.60E+07	1.30E+08		
148	3.90E+07	1.10E+07		7.00E+07	1.70E+07	

Composite SSF sample bench-scale data (P950425CF)
(Figure 11)

Flash #	← 1 (10 IU/g)				2 (10 IU/g)				3 (25 IU/g)			
Time (h)	Ethanol (g/L)	Lactic (g/L)	Acetic (g/L)	Glucose (g/L)	Ethanol (g/L)	Lactic (g/L)	Acetic (g/L)	Glucose (g/L)	Ethanol (g/L)	Lactic (g/L)	Acetic (g/L)	Glucose (g/L)
0	0.96				0.96				0.96			
87	16.45	2.28	3.56	3.12	18.74	2.49	4.35	3.35	25.06	2.25	3.79	**2.83**
110	19.31	2.2	3.68	3.8	20.53	2.28	**3.60**	3.2	25.86	2.5	4.66	2.87
136	22.61	2.55	5.07	3.35	22.39	2.51	4.51	2.68	26.26	2.22	4.17	1.92
159	23.71	2.5	5.13	2.84	23.69	2.56	4.7	2.61	27.3	2.52	5.36	2.84
209	24.05	2.37	5.3	1.94	23.83	2.47	5.14	2.1	27.52	2.58	6.43	2.82
231	25.78	2.49	5.7	2.18	26.13	2.54	5.89	2.96	29.06	2.67	7.34	3.24

Average of two flasks

	10IU/g				25IU/g			
	Ethanol (g/L)	Lactic (g/L)	Acetic (g/L)	Glucose (g/L)	Ethanol (g/L)	Lactic (g/L)	Acetic (g/L)	Glucose (g/L)
0	0.96				0.96			
87	17.595	2.385	3.955	3.235	24.96	2.375	4.13	2.97
110	19.92	2.24	3.68	3.5	25.355	2.44	4.53	2.755
I36	22.5	2.53	4.79	3.015	26.19	2.355	4.45	2.335
159	23.7	2.53	4.915	2,725	27.23	2.525	5,355	2.44
209	23.94	2.42	5.22	2.02	27.23	2.525	6.215	2.32
231	25.955	2.515	5.795	2.57	28.845	2.64	7.105	3.195

Flash #		4 (25 IU/g)		
Time (h)	Ethanol (g/L)	Lactic (g/L)	Acetic (g/L)	Glucose (g/L)
0	0.96			
87	24.86	*2.5*	4.47	3.11
110	24.85	*2.38*	4.4	2.64
136	26.12	2.49	4.73	2.75
159	27.16	**2.53**	5.35	2.04
209	26.94	*2.47*	6	1.82
231	28.63	2.61	*6.87*	3.15